Microarray1, 2, RNA seq datasets combined

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12/20/2021

```
library("mogene10sttranscriptcluster.db")
```

```
## Loading required package: AnnotationDbi

## Loading required package: stats4

## Loading required package: BiocGenerics

## Loading required package: parallel

##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, 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

## 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")'.
```

```
## Loading required package: IRanges

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: org.Mm.eg.db

##

##
```

```
library("ArrayExpress")
library("arrayQualityMetrics")
library("ggplot2")
library("huex10sttranscriptcluster.db")
```

```
## Loading required package: org.Hs.eg.db

##

##
```

```
library("limma")
```

```
##
## Attaching package: 'limma'

## The following object is masked from 'package:BiocGenerics':
##
##     plotMA
```

```
library("oligo")
```

```
## Loading required package: oligoClasses

## Welcome to oligoClasses version 1.54.0

## Loading required package: Biostrings

## Loading required package: XVector

## Loading required package: GenomeInfoDb
```

```
##
## Attaching package: 'Biostrings'

## The following object is masked from 'package:base':
##
##     strsplit

## ========================================================================

## Welcome to oligo version 1.56.0

## ========================================================================

##
## Attaching package: 'oligo'

## The following object is masked from 'package:limma':
##
##     backgroundCorrect
```

```
library("siggenes")
```

```
## Loading required package: multtest

## Loading required package: splines
```

```
library("affy")
```

```
##
## Attaching package: 'affy'

## The following objects are masked from 'package:oligo':
##
##     intensity, MAplot, mm, mm<-, mmindex, pm, pm<-, pmindex,
##     probeNames, rma

## The following object is masked from 'package:oligoClasses':
##
##     list.celfiles
```

```
#library("pd.huex.1.0.st.v2")
library("wateRmelon")
```

```
## Loading required package: matrixStats

##
## Attaching package: 'matrixStats'
```

```
## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians

## Loading required package: methylumi

## Loading required package: scales

## Loading required package: reshape2

## Loading required package: FDb.InfiniumMethylation.hg19

## Loading required package: GenomicFeatures

## Loading required package: GenomicRanges

## Loading required package: TxDb.Hsapiens.UCSC.hg19.knownGene

## Loading required package: minfi

## Loading required package: SummarizedExperiment

## Loading required package: MatrixGenerics

##
## 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

## The following object is masked from 'package:Biobase':
##
##     rowMedians

## Loading required package: bumphunter
```

```
## Loading required package: foreach

## Parallel computing support for 'oligo/crlmm': Disabled
##      - Load 'ff'
##      - Load and register a 'foreach' adaptor
##         Example - Using 'multicore' for 2 cores:
##              library(doMC)
##              registerDoMC(2)
## ================================================================================
## Loading required package: iterators
## Loading required package: locfit
## locfit 1.5-9.4    2020-03-24
## Setting options('download.file.method.GEOquery'='auto')
## Setting options('GEOquery.inmemory.gpl'=FALSE)
##
## Attaching package: 'minfi'
##
## The following object is masked from 'package:oligo':
##
##     getProbeInfo
##
## The following object is masked from 'package:oligoClasses':
##
##     getM
##
## Loading required package: lumi
## No methods found in package 'RSQLite' for request: 'dbListFields' when loading 'lumi'
##
## Attaching package: 'lumi'
##
## The following objects are masked from 'package:methylumi':
##
##     estimateM, getHistory
##
## The following objects are masked from 'package:affy':
##
##     MAplot, plotDensity
##
## The following object is masked from 'package:oligo':
##
##     MAplot
##
## Loading required package: ROC
## Loading required package: IlluminaHumanMethylation450kanno.ilmn12.hg19
## Loading required package: illuminaio
```

```
library("affy")
library("arrayQualityMetrics")
library("ArrayExpress")
library("RSQLite")
library("DBI")
library("htmltools")
library("biomaRt")
library("tximport")
```

```
library("edgeR")
library("rhdf5")
```

```
setwd("/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Dataset
```

#E-GEOD-57452

General info

The array used for this dataset is A-AFFY-130 - Affymetrix GeneChip Mouse Gene 1.0 ST Array [MoGene-1_0-st-v1]. Mice were infected with influenza and RNA was extracted from the lungs after 10 days. We used samples involving susceptible mice after 10 days of infection with influenza from this dataset.

Intensity values

Read in the microarray data and display the head and dimensions of the intensity value matrix.

```
id_1 <- "E-GEOD-57452"
exonCELs <- list.celfiles("../Datasets/Microarray1/")
data.raw_1 <- read.celfiles(paste(rep("../Datasets/Microarray1/",length(exonCELs)),exonCELs,sep=""))
```

```
## Reading in : ../Datasets/Microarray1/GSM1382971_lung_T-9_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382972_lung_T-9_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382973_lung_T-9_rep3.CEL
## Reading in : ../Datasets/Microarray1/GSM1382974_lung_T0_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382975_lung_T1_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382976_lung_T2_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382977_lung_T3_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382978_lung_T3_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382979_lung_T4_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382980_lung_T4_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382981_lung_T5_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382982_lung_T5_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382983_lung_T5_rep3.CEL
## Reading in : ../Datasets/Microarray1/GSM1382984_lung_T6_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382985_lung_T7_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382986_lung_T7_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382987_lung_T8_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382988_lung_T8_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382989_lung_T9_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382990_lung_T9_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382991_lung_T10_rep1.CEL
## Reading in : ../Datasets/Microarray1/GSM1382992_lung_T10_rep2.CEL
## Reading in : ../Datasets/Microarray1/GSM1382993_lung_T10_rep3.CEL
```

```
# make vector containing the sample class
samples <- c(replicate(3, "control"), "day0", "day1", "day2", replicate(2, "day3"), replicate(2, "day4"

# add the sample classes to the pData object
pData(data.raw_1)[,2] <- samples
```

```r
colnames(pData(data.raw_1)) <- c("index", "treatment")


# filter control samples and samples taken after 3 days of infection
filter <- colnames(data.raw_1)[data.raw_1@phenoData@data$treatment=="control" | data.raw_1@phenoData@da

# apply filter
filtered <- data.raw_1[,filter]

# check dimentsions of filtered object
dim(exprs(filtered))
```

```
## [1] 1102500       6
```

```r
## arrayQualityMetrics
#arrayQualityMetrics(filtered,outdir="../Datasets/microarray1/raw1",force=T)
#arrayQualityMetrics(filtered,outdir="../Datasets/microarray1/rawlog1",force=T,do.logtransform=T)

# Preprocessing (using the oligo function because affy didnt work)
MouseRMA<- oligo::rma(filtered,background=T)
```

```
## Background correcting
## Normalizing
## Calculating Expression
```

```r
## QC post preprocessing
#arrayQualityMetrics(MouseRMA,outdir="../Datasets/microarray1/rma1",force=T)            #RMA produces l
```

##Data exploration

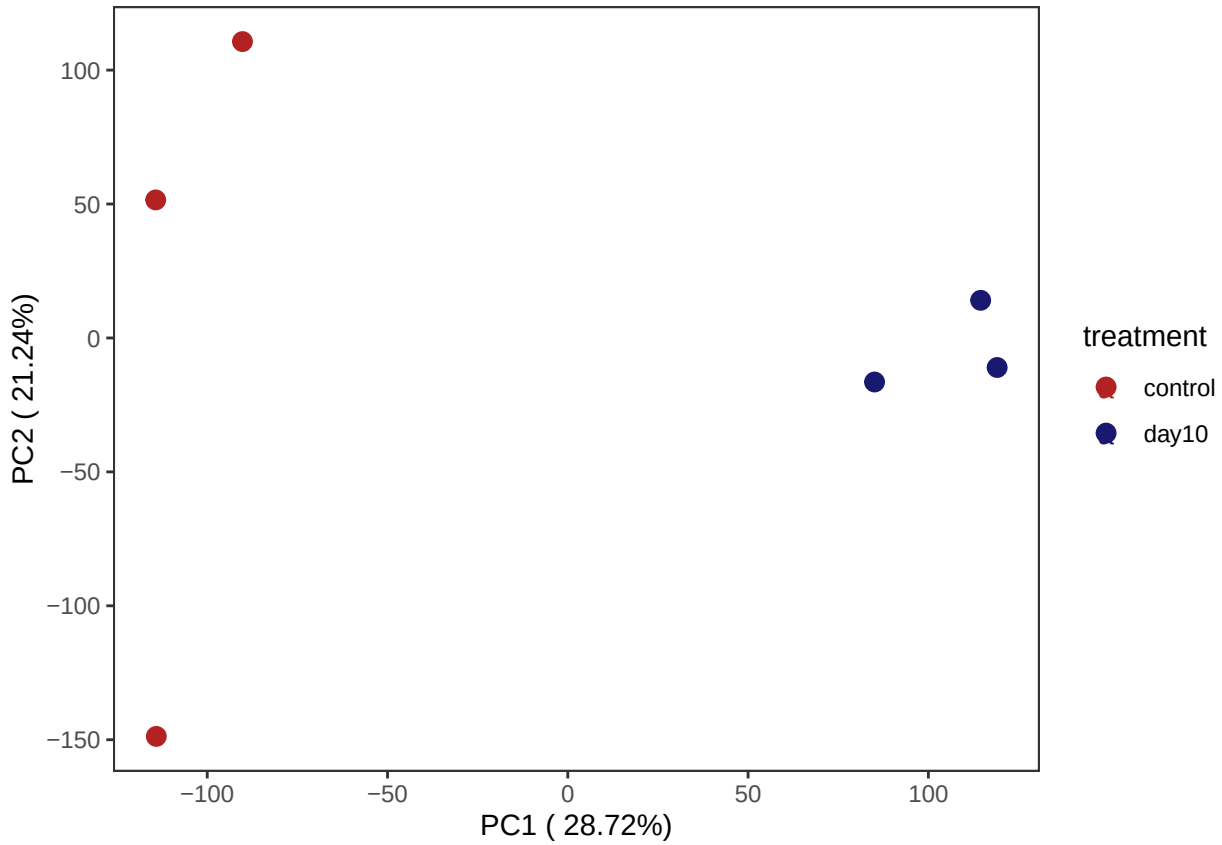
```r
# transpose the data before PcA as this function requires the variables to b columns
data <- t(as.data.frame(MouseRMA@assayData$exprs))
pca <- prcomp(data, center = T, scale. = T)

summary(pca)
```

```
## Importance of components:
##                             PC1     PC2     PC3     PC4      PC5       PC6
## Standard deviation     117.2632 86.7380 83.3066 63.5689 57.45316 6.844e-13
## Proportion of Variance   0.3867  0.2116  0.1952  0.1137  0.09284 0.000e+00
## Cumulative Proportion    0.3867  0.5983  0.7935  0.9072  1.00000 1.000e+00
```

```r
# save as dataframe and add treatment variable
pca_out <- as.data.frame(pca$x)
pca_out$treatment <- as.character(MouseRMA@phenoData@data$treatment)

# get lablels
percentage <- round(pca$sdev / sum(pca$sdev) * 100, 2)
percentage <- paste( colnames(pca_out), "(", paste( as.character(percentage), "%", ")", sep="") )

ggplot(data = pca_out)+
```

```
  geom_point(aes(x = PC1, y = PC2, colour = treatment, label=''), size=3)+
  geom_text(aes(x = PC1, y = PC2, colour = treatment, label=''),hjust=0.5, vjust=1.15)+
  theme_bw()+
  xlab(percentage[1])+
  ylab(percentage[2])+
  labs(colour = "treatment")+
  theme(plot.title = element_text(hjust = 0.5))+
  scale_colour_manual(values = c("firebrick", "midnightblue"))+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
```

```
## Warning: Ignoring unknown aesthetics: label
```

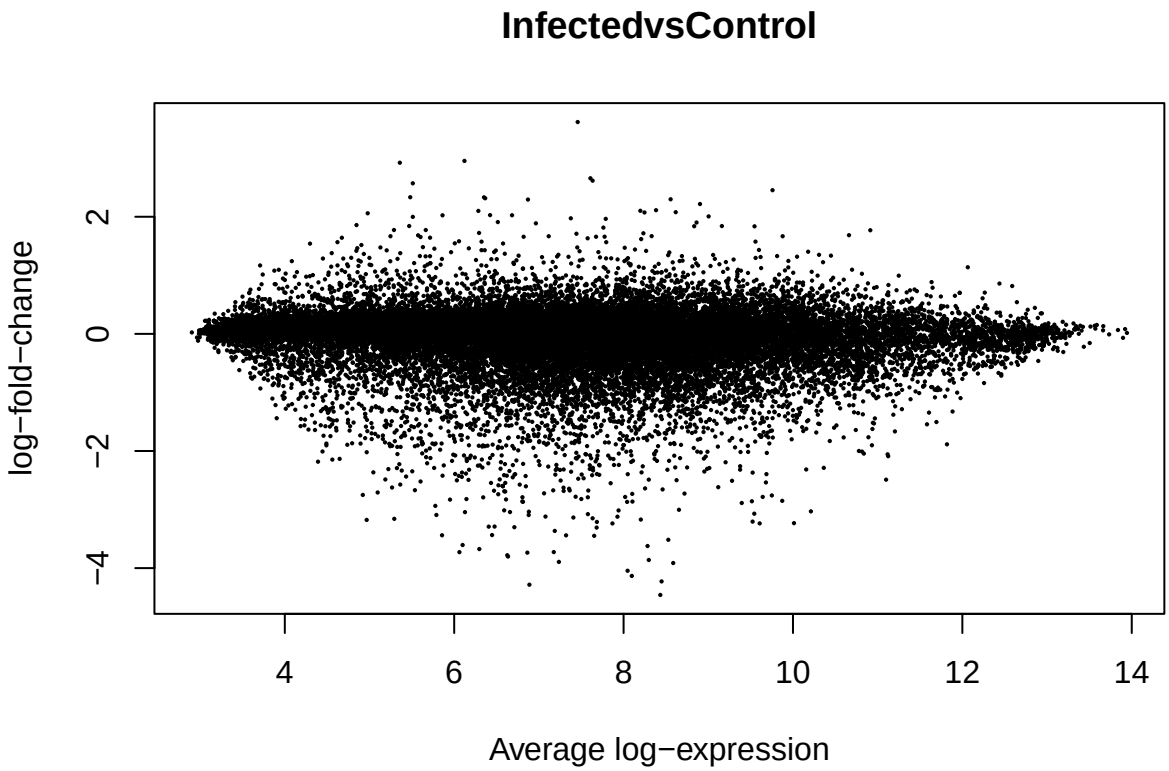



```
ggsave("PCA_array1.png", dpi=750, width=8, height = 5)
```

determine differential expression

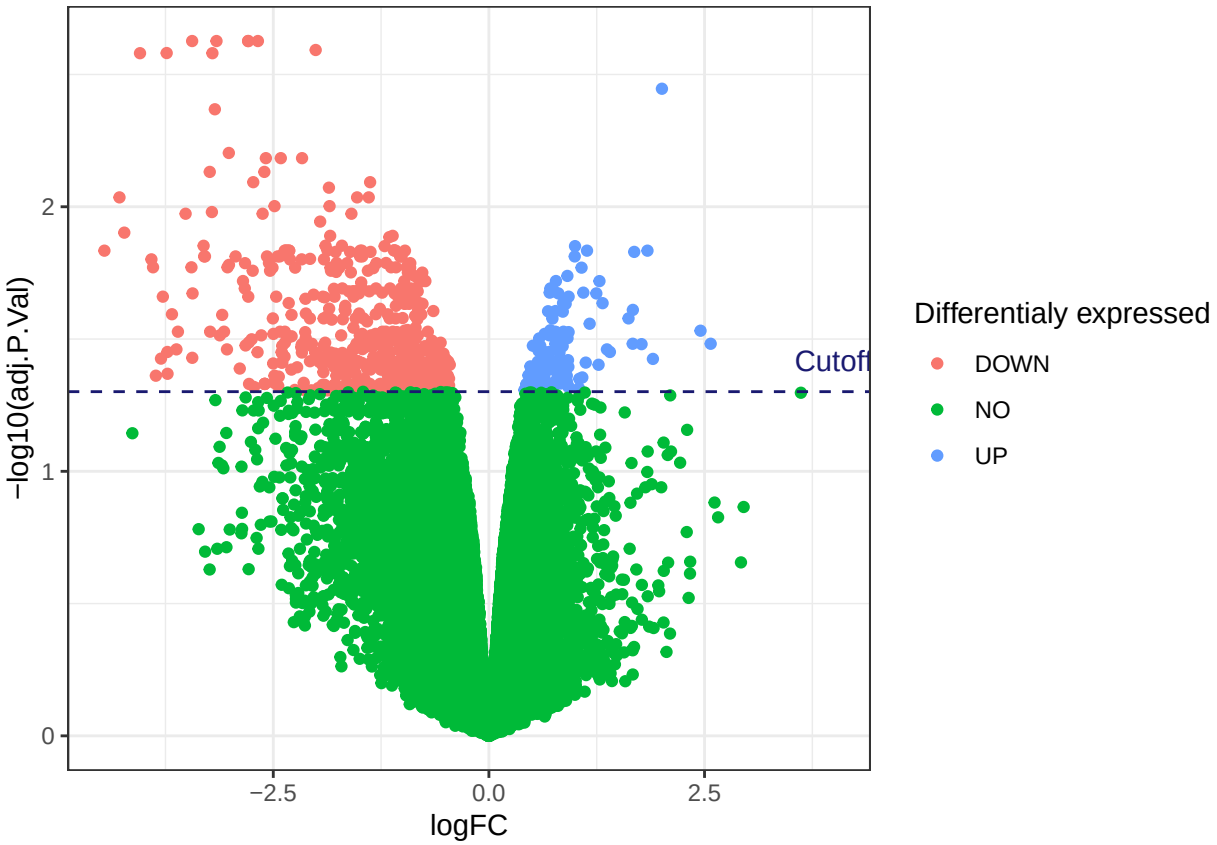
```
annot <- factor(pData(MouseRMA)[,2])

## Differential expression by LIMMA
# Method as stated in limma package (no intercept, easy for simple model designs)
design <- model.matrix(~0+annot)
colnames(design)<-c("control","infected")

# make linear model
```

```
fit <- lmFit(MouseRMA,design)

# create contrast matrix to get the differential expression between samples from infected mice and unin
cont.matrix <- makeContrasts(InfectedvsControl=control-infected,levels=design)
fit2 <- contrasts.fit(fit,cont.matrix)
fit2 <- eBayes(fit2)

# make MA plot for model with applied contrast matrix
limma::plotMA(fit2)
```




```
library(ggplot2)
# DE results with multiple testing correction (Benjamini-Hochberg = BH)
LIMMAout <- topTable(fit2,adjust="BH",number=nrow(exprs(MouseRMA)))



# add column indicating for all differntially expressed genes (adjusted p-value < 0.05) whether they're

LIMMAout$diffexpressed <- "NO"
LIMMAout$diffexpressed[LIMMAout$logFC > 0 & LIMMAout$adj.P.Val < 0.05] <- "UP"
LIMMAout$diffexpressed[LIMMAout$logFC < 0 & LIMMAout$adj.P.Val < 0.05] <- "DOWN"

# do the same but if we would not correct for multiple testing.
LIMMAout$diffexpressed_no_BH <- "NO"
LIMMAout$diffexpressed_no_BH[LIMMAout$logFC > 0 & LIMMAout$P.Value < 0.05] <- "UP"
```

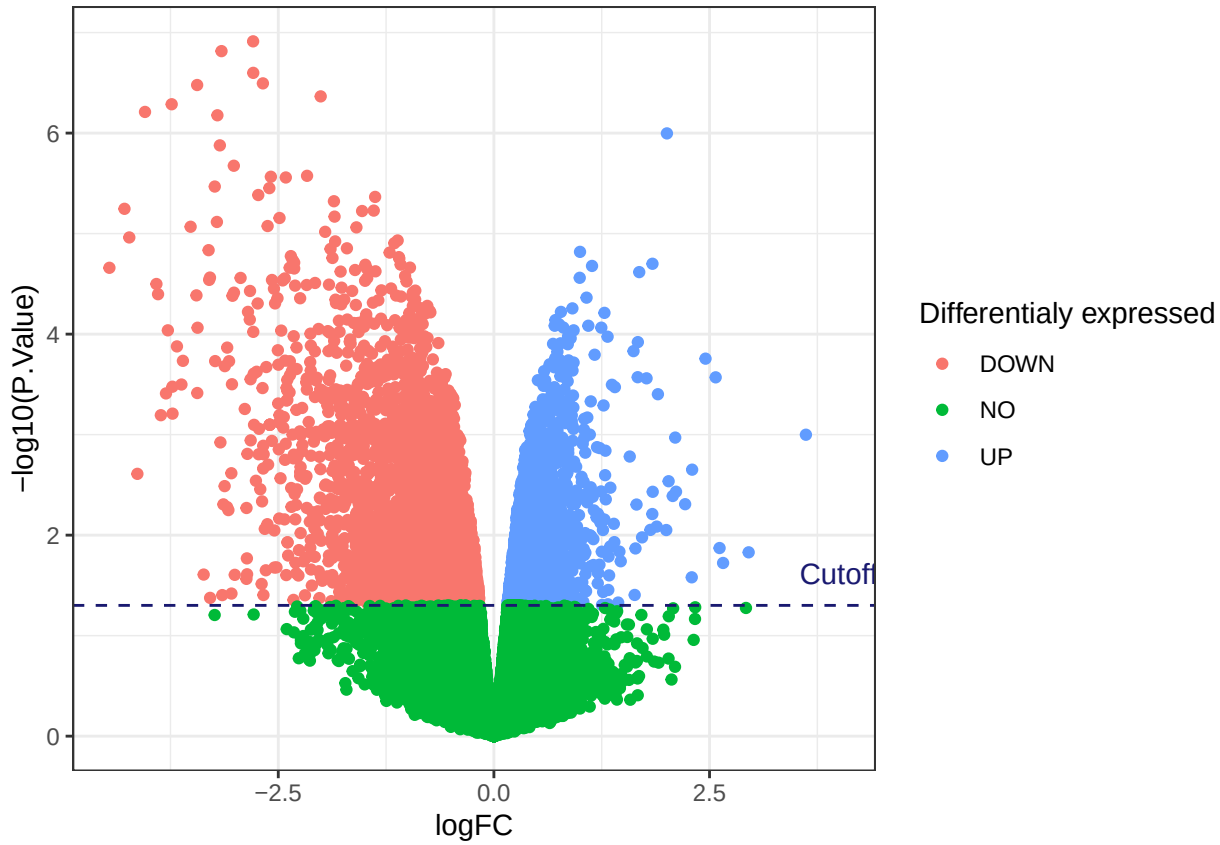
```
LIMMAout$diffexpressed_no_BH[LIMMAout$logFC < 0 & LIMMAout$P.Value < 0.05] <- "DOWN"

# code to make volcano plots

ggplot(data = LIMMAout, aes(x= logFC, y = -log10(adj.P.Val), colour = diffexpressed)) +
 geom_point()+
 theme_bw()+
  geom_hline(yintercept = -log10(0.05), linetype="dashed", color="midnightblue")+
  annotate("text", min(4), 1.3, vjust = -1, label = "Cutoff", color="midnightblue")+
 labs(colour = "Differentialy expressed")
```




```
ggsave("volcanoplot.png", dpi=750)
```

```
## Saving 6.5 x 4.5 in image
```

```
ggplot(data = LIMMAout, aes(x= logFC, y = -log10(P.Value), colour = diffexpressed_no_BH)) +
 geom_point()+
 theme_bw()+
  geom_hline(yintercept = -log10(0.05), linetype="dashed", color="midnightblue")+
  annotate("text", min(4), 1.3, vjust = -1, label = "Cutoff", color="midnightblue")+
 labs(colour = "Differentialy expressed")
```




```
table(LIMMAout$diffexpressed)
```

```
##
##  DOWN    NO    UP
##   570 34863   123
```

Annotation

```
# get the annotation trough package (mogene10sttranscriptcluster.db) found at https://www.biostars.org/
columns(mogene10sttranscriptcluster.db)
```

```
##  [1] "ACCNUM"       "ALIAS"        "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS"
##  [6] "ENTREZID"     "ENZYME"       "EVIDENCE"     "EVIDENCEALL"  "GENENAME"
## [11] "GENETYPE"     "GO"           "GOALL"        "IPI"          "MGI"
## [16] "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"         "PMID"
## [21] "PROBEID"      "PROSITE"      "REFSEQ"       "SYMBOL"       "UNIPROT"
```

```
annotTable <- select(
  mogene10sttranscriptcluster.db,
  keys = keys(mogene10sttranscriptcluster.db),
  column = c('PROBEID', 'SYMBOL', 'ENTREZID', 'ENSEMBL', 'GENENAME', 'PROSITE'),
  keytype = 'PROBEID')
```

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

```
## sort annotation data alphabetically on probe name

annotTable.filt <- annotTable[sort(annotTable$PROBEID,index.return=T)$ix,]

# merge information from multiple lines describing the same probe
probe <- "start"
position <- 0
for (i in 1:dim(annotTable.filt)[1]){
  if (annotTable.filt[i, 1] != probe){
    probe <- annotTable.filt[i, 1]
    position <- i
  }
  else{
    # concatenate the information o f the 2 lines with a ; as separator
    annotTable.filt[position,2:5] <- paste(annotTable.filt[position,2:5], annotTable.filt[i, 2:5], sep=
    # mark the line
    annotTable.filt[i,1] <- NA
  }
}

annotTable.filt <- annotTable.filt[!is.na(annotTable.filt$PROBEID),]

## Check if all probes are present in both sets
dim(annotTable.filt)
```

```
## [1] 35556     6
```

```
dim(LIMMAout)
```

```
## [1] 35556     8
```

```
 ## Double check => "Assumption is the mother of all fuck up's ;)"
sum(annotTable.filt$PROBEID!=sort(rownames(LIMMAout)))
```

```
## [1] 0
```

```
## Sort LIMMA output alphabetically on probe name
LIMMAout_sorted <- LIMMAout[sort(rownames(LIMMAout),index.return=T)$ix,]


## Add gene names to LIMMA output
LIMMAout_sorted$gene <- annotTable.filt$SYMBOL
LIMMAout_annot <- LIMMAout_sorted[sort(LIMMAout_sorted$adj.P.Val,index.return=T)$ix,]

# determine how many differentially expressed probes have an annotated gene
table(is.na(LIMMAout_annot[LIMMAout_annot$diffexpressed != "NO",9]))
```

```
##
## FALSE  TRUE
##   510   183
```

```r
## alternative annotation method:

# annotation file from https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL6246
annotation_MA <- read.delim("./Microarray1/GPL6246.annot",header=T, sep="\t", skip=27, fill=T)
print(head(annotation_MA))
```

```
##         ID           Gene.title Gene.symbol   Gene.ID UniGene.title
## 1 10344614  predicted gene 2889      Gm2889 100040658
## 2 10344616
## 3 10344618
## 4 10344620 predicted gene 10568     Gm10568 100038431
## 5 10344622
## 6 10344624  lysophospholipase 1      Lypla1     18777
##   UniGene.symbol UniGene.ID
## 1
## 2
## 3
## 4
## 5
## 6
##
## 1
## 2
## 3
## 4
## 5
## 6 Mus musculus lysophospholipase 1 (Lypla1), mRNA///PREDICTED: Mus musculus lysophospholipase 1 (Lyp
##
## 1                                                                                                74211482/
## 2
## 3
## 4
## 5
## 6 31543132///1039727931///26341311///74191027///15488807///31127306///71059730///1864158///12854598/
##                                                                                                    GenBa
## 1                                                                                                 AK14551
## 2
## 3
## 4
## 5
## 6 NM_008866///XM_006495471///AK050549///AK167231///BC013536///BC052848///CT010201///U89352///AK01602
##   Platform_CLONEID Platform_ORF      Platform_SPOTID Chromosome.location
## 1               NA           NA chr1:3054233-3054733                  18
## 2               NA           NA chr1:3102016-3102125
## 3               NA           NA chr1:3276323-3277348
## 4               NA           NA chr1:3680571-3680912
## 5               NA           NA chr1:4771131-4772199
## 6               NA           NA chr1:4807862-4846736               1 A1
##                         Chromosome.annotation
## 1                                 Chromosome 18
## 2
## 3
## 4                                  Chromosome 1
```

```
## 5
## 6 Chromosome 1, NC_000067.6 (4807560..4846739)
##
## 1
## 2
## 3
## 4
## 5
## 6 2,4,4-trimethyl-3-oxopentanoyl-CoA thioesterase activity///2-oxoglutaryl-CoA thioesterase activity,
##
## 1
## 2
## 3
## 4
## 5
## 6 fatty acid metabolic process///lipid metabolic process///negative regulation of Golgi to plasma mem
##                                                    GO.Component
## 1
## 2
## 3
## 4
## 5
## 6 cytoplasm///cytosol///extracellular exosome///mitochondrion
##
## 1
## 2
## 3
## 4
## 5
## 6 GO:0034869///GO:0034843///GO:0034946///GO:0016289///GO:0047617///GO:0052689///GO:0044466///GO:0016
##                                       GO.Process.ID
## 1
## 2
## 3
## 4
## 5
## 6 GO:0006631///GO:0006629///GO:0042997///GO:0002084
##                                     GO.Component.ID
## 1
## 2
## 3
## 4
## 5
## 6 GO:0005737///GO:0005829///GO:0070062///GO:0005739
```

```
annotation_MA <- annotation_MA[sort(annotation_MA$ID,index.return=T)$ix,]

dim(annotation_MA)
```

```
## [1] 35558    21
```

```
dim(LIMMAout)
```

```
## [1] 35556     8
```

```
## the dimensions don't match but we can still check whether the information for the differentially exp

#annotation_MA[annotation_MA$ID %in% rownames(LIMMAout_annot[LIMMAout_annot$diffexpressed != "NO",]),]
```

```
#get DE genes symbols
DEgenes_symbols1 <- unique(annotation_MA[annotation_MA$ID %in% rownames(LIMMAout_annot[LIMMAout_annot$di
             & annotation_MA$Gene.symbol != "",]$Gene.symbol)
head(DEgenes_symbols1)
```

```
## [1] "Cspp1"  "Cetn4"  "Il1r2"  "Il18r1" "Ormdl1" "Nif3l1"
```

Microarray2: E-GEOD-64750

General info

The array used for this dataset is A-AFFY-45 - Affymetrix GeneChip Mouse Genome 430 2.0 [Mouse430_2]. In this experiment, susceptible mice were infected with H5N1 influenza. After 72h RNA was extracted from the lungs of the mice. We used 9 samples of this experiment (susceptible mice).

Intensity values

Read in the microarray data and examine dimensioniality of the intensity value matrix.

```
id <- "E-GEOD-64750"
exonCELs <- list.celfiles("../Datasets/Microarray2/")
data.raw_2 <- read.celfiles(paste(rep("../Datasets/Microarray2/",length(exonCELs)),exonCELs,sep=""))
```

```
## Reading in : ../Datasets/Microarray2/GSM1579245_jac013-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579246_jac021-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579247_jac044-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579248_jac045-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579249_jac046-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579250_jac007-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579251_jac008-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579252_jac036-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579253_jac047-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579254_jac014-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579255_jac024-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579256_jac025-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579257_jac011-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579258_jac012-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579259_jac037-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579260_jac048-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579261_jac015-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579262_jac016-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579263_jac017-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579264_jac018-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579265_jac019-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579266_jac020-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579267_jac027-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579268_jac028-430v2.CEL
```

```
## Reading in : ../Datasets/Microarray2/GSM1579269_jac034-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579270_jac033-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579271_jac029-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579272_jac035-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579273_jac041-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579274_jac042-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579275_jac043-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579276_jac030-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579277_jac031-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579278_jac032-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579279_jac038-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579280_jac039-430v2.CEL
## Reading in : ../Datasets/Microarray2/GSM1579281_jac040-430v2.CEL
```

```
dim(exprs(data.raw_2))
```

```
## [1] 1004004      37
```

```
head(exprs(data.raw_2))
```

```
##   GSM1579245_jac013-430v2.CEL GSM1579246_jac021-430v2.CEL
## 1                          62                         191
## 2                        5707                       16272
## 3                          69                         265
## 4                        5913                       16107
## 5                          57                         143
## 6                          54                         143
##   GSM1579247_jac044-430v2.CEL GSM1579248_jac045-430v2.CEL
## 1                         198                         134
## 2                        7485                        5697
## 3                         200                         144
## 4                        7664                        6184
## 5                         154                         121
## 6                         162                         128
##   GSM1579249_jac046-430v2.CEL GSM1579250_jac007-430v2.CEL
## 1                         136                         123
## 2                        6036                        7786
## 3                         134                         162
## 4                        6147                        7832
## 5                         126                         111
## 6                         113                         105
##   GSM1579251_jac008-430v2.CEL GSM1579252_jac036-430v2.CEL
## 1                         139                          80
## 2                        8743                        6310
## 3                         154                         102
## 4                        8940                        6335
## 5                         102                          70
## 6                          96                          88
##   GSM1579253_jac047-430v2.CEL GSM1579254_jac014-430v2.CEL
## 1                          92                         105
## 2                        4988                        8341
## 3                          87                         159
## 4                        5275                        8721
```

```
## 5                          87                          109
## 6                          79                           96
##   GSM1579255_jac024-430v2.CEL GSM1579256_jac025-430v2.CEL
## 1                         125                          153
## 2                        9656                         8775
## 3                         200                          180
## 4                       10228                         8859
## 5                          94                          101
## 6                         118                          121
##   GSM1579257_jac011-430v2.CEL GSM1579258_jac012-430v2.CEL
## 1                         182                          130
## 2                       13154                         9498
## 3                         218                          195
## 4                       13689                         9705
## 5                         104                          108
## 6                         132                          128
##   GSM1579259_jac037-430v2.CEL GSM1579260_jac048-430v2.CEL
## 1                         176                           92
## 2                       15363                         4973
## 3                         251                           72
## 4                       15737                         5058
## 5                         142                           72
## 6                         173                           63
##   GSM1579261_jac015-430v2.CEL GSM1579262_jac016-430v2.CEL
## 1                          59                           69
## 2                        5959                         6331
## 3                          73                           72
## 4                        6140                         6388
## 5                          66                           65
## 6                          51                           71
##   GSM1579263_jac017-430v2.CEL GSM1579264_jac018-430v2.CEL
## 1                          58                           69
## 2                        5856                         6670
## 3                          78                           82
## 4                        5730                         6455
## 5                          61                           92
## 6                          59                           47
##   GSM1579265_jac019-430v2.CEL GSM1579266_jac020-430v2.CEL
## 1                          87                          202
## 2                        6029                         6455
## 3                          78                           74
## 4                        6409                         6648
## 5                          65                          102
## 6                          67                           58
##   GSM1579267_jac027-430v2.CEL GSM1579268_jac028-430v2.CEL
## 1                         161                          123
## 2                        7684                         8069
## 3                         230                          156
## 4                        7162                         8064
## 5                         123                           97
## 6                         139                          146
##   GSM1579269_jac034-430v2.CEL GSM1579270_jac033-430v2.CEL
## 1                         193                          150
## 2                       14103                         7298
```

```
## 3                          279                          231
## 4                        14199                         7447
## 5                          156                          145
## 6                          197                          139
##   GSM1579271_jac029-430v2.CEL GSM1579272_jac035-430v2.CEL
## 1                          242                          149
## 2                        18215                         7908
## 3                          283                          150
## 4                        17438                         8798
## 5                          160                          137
## 6                          151                          141
##   GSM1579273_jac041-430v2.CEL GSM1579274_jac042-430v2.CEL
## 1                          132                          184
## 2                         8993                         8146
## 3                          185                          169
## 4                         9202                         8344
## 5                          153                          180
## 6                          117                          152
##   GSM1579275_jac043-430v2.CEL GSM1579276_jac030-430v2.CEL
## 1                          187                          101
## 2                         6867                         6737
## 3                          223                           92
## 4                         7165                         6788
## 5                          101                           61
## 6                          138                           83
##   GSM1579277_jac031-430v2.CEL GSM1579278_jac032-430v2.CEL
## 1                           74                          165
## 2                         7379                         7416
## 3                           67                          201
## 4                         7312                         7929
## 5                           65                          157
## 6                           77                          141
##   GSM1579279_jac038-430v2.CEL GSM1579280_jac039-430v2.CEL
## 1                           72                           72
## 2                         4883                         4924
## 3                           68                           74
## 4                         4814                         4988
## 5                           68                           65
## 6                           71                           57
##   GSM1579281_jac040-430v2.CEL
## 1                           81
## 2                         4475
## 3                           78
## 4                         4646
## 5                           59
## 6                           67
```

Annotation

Here we provide basic sample annotation, including the phenotype of interest and relevant other features (e.g. confounders). This dataset contains array data (A-AFFY-45) of different mice strains (BXD98, BXD97, BXD83, BXD73, BXD68, BXD67 ,BXD43, C57BL/6J, DBA/2J) infected with influenza virus H5N1.

```
sdrf <- read.delim("./Microarray2/E-GEOD-64750.sdrf.txt")
print(sdrf[,c("Source.Name","Comment..Sample_source_name.","Array.Design.REF", "Characteristics..strain
```

```
##     Source.Name                                  Comment..Sample_source_name.
## 1  GSM1579281 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 2  GSM1579280 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 3  GSM1579279 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 4  GSM1579278 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 5  GSM1579277 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 6  GSM1579276 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 7  GSM1579275 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 8  GSM1579274 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 9  GSM1579273 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 10 GSM1579272 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 11 GSM1579271 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 12 GSM1579270 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 13 GSM1579269 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 14 GSM1579268 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 15 GSM1579267 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 16 GSM1579266 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 17 GSM1579265 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 18 GSM1579264 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 19 GSM1579263 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 20 GSM1579262 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 21 GSM1579261 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 22 GSM1579260 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 23 GSM1579259 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 24 GSM1579258 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 25 GSM1579257 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 26 GSM1579256 1                                                Uninfected control
## 27 GSM1579255 1                                                Uninfected control
## 28 GSM1579254 1                                                Uninfected control
## 29 GSM1579253 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 30 GSM1579252 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 31 GSM1579251 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 32 GSM1579250 1 Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## 33 GSM1579249 1                                                Uninfected control
## 34 GSM1579248 1                                                Uninfected control
## 35 GSM1579247 1                                                Uninfected control
## 36 GSM1579246 1                                                Uninfected control
## 37 GSM1579245 1                                                Uninfected control
##    Array.Design.REF Characteristics..strain.
## 1         A-AFFY-45                    BXD98
## 2         A-AFFY-45                    BXD98
## 3         A-AFFY-45                    BXD98
## 4         A-AFFY-45                    BXD97
## 5         A-AFFY-45                    BXD97
## 6         A-AFFY-45                    BXD97
## 7         A-AFFY-45                    BXD83
## 8         A-AFFY-45                    BXD83
## 9         A-AFFY-45                    BXD83
## 10        A-AFFY-45                    BXD73
## 11        A-AFFY-45                    BXD73
```

```
## 12        A-AFFY-45                   BXD73
## 13        A-AFFY-45                   BXD68
## 14        A-AFFY-45                   BXD68
## 15        A-AFFY-45                   BXD68
## 16        A-AFFY-45                   BXD67
## 17        A-AFFY-45                   BXD67
## 18        A-AFFY-45                   BXD67
## 19        A-AFFY-45                   BXD43
## 20        A-AFFY-45                   BXD43
## 21        A-AFFY-45                   BXD43
## 22        A-AFFY-45                 C57BL/6J
## 23        A-AFFY-45                 C57BL/6J
## 24        A-AFFY-45                 C57BL/6J
## 25        A-AFFY-45                 C57BL/6J
## 26        A-AFFY-45                 C57BL/6J
## 27        A-AFFY-45                 C57BL/6J
## 28        A-AFFY-45                 C57BL/6J
## 29        A-AFFY-45                   DBA/2J
## 30        A-AFFY-45                   DBA/2J
## 31        A-AFFY-45                   DBA/2J
## 32        A-AFFY-45                   DBA/2J
## 33        A-AFFY-45                   DBA/2J
## 34        A-AFFY-45                   DBA/2J
## 35        A-AFFY-45                   DBA/2J
## 36        A-AFFY-45                   DBA/2J
## 37        A-AFFY-45                   DBA/2J
##                                                                                Comment..Sample_description.
## 1  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 2  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 3  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 4  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 5  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 6  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 7  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 8  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 9  Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 10 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 11 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 12 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 13 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 14 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 15 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 16 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 17 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 18 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 19 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 20 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 21 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 22 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 23 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 24 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 25 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 26                                          Gene expression data from lungs of unfected mice
## 27                                          Gene expression data from lungs of unfected mice
```

```
## 28                                          Gene expression data from lungs of unfected mice
## 29 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 30 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 31 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 32 Gene expression data from lungs of highly pathogenic H5N1 influenza A virus infected mice
## 33                                          Gene expression data from lungs of unfected mice
## 34                                          Gene expression data from lungs of unfected mice
## 35                                          Gene expression data from lungs of unfected mice
## 36                                          Gene expression data from lungs of unfected mice
## 37                                          Gene expression data from lungs of unfected mice
```

###Which samples we are using, and not using: We will be using samples involving susceptible and resistant mouse strain, DBA/2J (GSM1579245 - GSM1579253) and C57BL/6J (GSM1579254 - GSM1579260) respectively. Each strain was inoculated with H5N1 influenza A virus. We are not using the data from other strains (BXD98, BXD97, BXD83, BXD73, BXD68, BXD67, BXD43) which do not contain non-infected control samples.

```
#Load in the ExpressionFeatureSet object
setwd("/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Dataset
MouseExp_AE2 <- ArrayExpress("E-GEOD-64750")
```

```
## Copying raw data files

## Unpacking data files

## ArrayExpress: Reading pheno data from SDRF

## ArrayExpress: Reading data files

## Platform design info loaded.

## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579281_jac040-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579280_jac039-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579279_jac038-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579278_jac032-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579277_jac031-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579276_jac030-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579275_jac043-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579274_jac042-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579273_jac041-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579272_jac035-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579271_jac029-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579270_jac033-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579269_jac034-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579268_jac028-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579267_jac027-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579266_jac020-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579265_jac019-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579264_jac018-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579263_jac017-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579262_jac016-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579261_jac015-430v2.CEL
```

```
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579260_jac048-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579259_jac037-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579258_jac012-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579257_jac011-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579256_jac025-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579255_jac024-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579254_jac014-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579253_jac047-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579252_jac036-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579251_jac008-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579250_jac007-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579249_jac046-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579248_jac045-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579247_jac044-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579246_jac021-430v2.CEL
## Reading in : /var/folders/my/9t5kkz3n4x15jqxc9cf6hy740000gn/T//RtmparjxFU/GSM1579245_jac013-430v2.CEL

##
##  E-GEOD-64750  was successfully loaded into  ExpressionFeatureSet
```

```
#Load in the Affybatch object
setwd("/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Dataset
MouseExp_pheno2 <- ReadAffy(phenoData=pData(MouseExp_AE2))
```

```
#load first lines of output from the object
head(exprs(MouseExp_pheno2))
```

```
##   GSM1579281_jac040-430v2.CEL GSM1579280_jac039-430v2.CEL
## 1                          81                          72
## 2                        4475                        4924
## 3                          78                          74
## 4                        4646                        4988
## 5                          59                          65
## 6                          67                          57
##   GSM1579279_jac038-430v2.CEL GSM1579278_jac032-430v2.CEL
## 1                          72                         165
## 2                        4883                        7416
## 3                          68                         201
## 4                        4814                        7929
## 5                          68                         157
## 6                          71                         141
##   GSM1579277_jac031-430v2.CEL GSM1579276_jac030-430v2.CEL
## 1                          74                         101
## 2                        7379                        6737
## 3                          67                          92
## 4                        7312                        6788
## 5                          65                          61
## 6                          77                          83
##   GSM1579275_jac043-430v2.CEL GSM1579274_jac042-430v2.CEL
## 1                         187                         184
## 2                        6867                        8146
## 3                         223                         169
## 4                        7165                        8344
```

```
## 5                          101                          180
## 6                          138                          152
##   GSM1579273_jac041-430v2.CEL GSM1579272_jac035-430v2.CEL
## 1                          132                          149
## 2                         8993                         7908
## 3                          185                          150
## 4                         9202                         8798
## 5                          153                          137
## 6                          117                          141
##   GSM1579271_jac029-430v2.CEL GSM1579270_jac033-430v2.CEL
## 1                          242                          150
## 2                        18215                         7298
## 3                          283                          231
## 4                        17438                         7447
## 5                          160                          145
## 6                          151                          139
##   GSM1579269_jac034-430v2.CEL GSM1579268_jac028-430v2.CEL
## 1                          193                          123
## 2                        14103                         8069
## 3                          279                          156
## 4                        14199                         8064
## 5                          156                           97
## 6                          197                          146
##   GSM1579267_jac027-430v2.CEL GSM1579266_jac020-430v2.CEL
## 1                          161                          202
## 2                         7684                         6455
## 3                          230                           74
## 4                         7162                         6648
## 5                          123                          102
## 6                          139                           58
##   GSM1579265_jac019-430v2.CEL GSM1579264_jac018-430v2.CEL
## 1                           87                           69
## 2                         6029                         6670
## 3                           78                           82
## 4                         6409                         6455
## 5                           65                           92
## 6                           67                           47
##   GSM1579263_jac017-430v2.CEL GSM1579262_jac016-430v2.CEL
## 1                           58                           69
## 2                         5856                         6331
## 3                           78                           72
## 4                         5730                         6388
## 5                           61                           65
## 6                           59                           71
##   GSM1579261_jac015-430v2.CEL GSM1579260_jac048-430v2.CEL
## 1                           59                           92
## 2                         5959                         4973
## 3                           73                           72
## 4                         6140                         5058
## 5                           66                           72
## 6                           51                           63
##   GSM1579259_jac037-430v2.CEL GSM1579258_jac012-430v2.CEL
## 1                          176                          130
## 2                        15363                         9498
```

```
## 3                          251                          195
## 4                        15737                         9705
## 5                          142                          108
## 6                          173                          128
##   GSM1579257_jac011-430v2.CEL GSM1579256_jac025-430v2.CEL
## 1                          182                          153
## 2                        13154                         8775
## 3                          218                          180
## 4                        13689                         8859
## 5                          104                          101
## 6                          132                          121
##   GSM1579255_jac024-430v2.CEL GSM1579254_jac014-430v2.CEL
## 1                          125                          105
## 2                         9656                         8341
## 3                          200                          159
## 4                        10228                         8721
## 5                           94                          109
## 6                          118                           96
##   GSM1579253_jac047-430v2.CEL GSM1579252_jac036-430v2.CEL
## 1                           92                           80
## 2                         4988                         6310
## 3                           87                          102
## 4                         5275                         6335
## 5                           87                           70
## 6                           79                           88
##   GSM1579251_jac008-430v2.CEL GSM1579250_jac007-430v2.CEL
## 1                          139                          123
## 2                         8743                         7786
## 3                          154                          162
## 4                         8940                         7832
## 5                          102                          111
## 6                           96                          105
##   GSM1579249_jac046-430v2.CEL GSM1579248_jac045-430v2.CEL
## 1                          136                          134
## 2                         6036                         5697
## 3                          134                          144
## 4                         6147                         6184
## 5                          126                          121
## 6                          113                          128
##   GSM1579247_jac044-430v2.CEL GSM1579246_jac021-430v2.CEL
## 1                          198                          191
## 2                         7485                        16272
## 3                          200                          265
## 4                         7664                        16107
## 5                          154                          143
## 6                          162                          143
##   GSM1579245_jac013-430v2.CEL
## 1                           62
## 2                         5707
## 3                           69
## 4                         5913
## 5                           57
## 6                           54
```

```
head(pData(MouseExp_pheno2))  #source name, comment sample description, sample source name, sample titl
```

```
##                               Source.Name
## GSM1579281_jac040-430v2.CEL GSM1579281 1
## GSM1579280_jac039-430v2.CEL GSM1579280 1
## GSM1579279_jac038-430v2.CEL GSM1579279 1
## GSM1579278_jac032-430v2.CEL GSM1579278 1
## GSM1579277_jac031-430v2.CEL GSM1579277 1
## GSM1579276_jac030-430v2.CEL GSM1579276 1
##                                                                                 Comment..Sa
## GSM1579281_jac040-430v2.CEL Gene expression data from lungs of highly pathogenic H5N1 influenza A vi
## GSM1579280_jac039-430v2.CEL Gene expression data from lungs of highly pathogenic H5N1 influenza A vi
## GSM1579279_jac038-430v2.CEL Gene expression data from lungs of highly pathogenic H5N1 influenza A vi
## GSM1579278_jac032-430v2.CEL Gene expression data from lungs of highly pathogenic H5N1 influenza A vi
## GSM1579277_jac031-430v2.CEL Gene expression data from lungs of highly pathogenic H5N1 influenza A vi
## GSM1579276_jac030-430v2.CEL Gene expression data from lungs of highly pathogenic H5N1 influenza A vi
##                                                    Comment..Sample_source_name.
## GSM1579281_jac040-430v2.CEL Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## GSM1579280_jac039-430v2.CEL Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## GSM1579279_jac038-430v2.CEL Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## GSM1579278_jac032-430v2.CEL Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## GSM1579277_jac031-430v2.CEL Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
## GSM1579276_jac030-430v2.CEL Highly Pathogenic H5N1 Influenza A virus infected; 72 hours
##
## GSM1579281_jac040-430v2.CEL BXD98 Recombinant inbred mouse strain. Lung homogenate 72 hours post H5N
## GSM1579280_jac039-430v2.CEL BXD98 Recombinant inbred mouse strain. Lung homogenate 72 hours post H5N
## GSM1579279_jac038-430v2.CEL BXD98 Recombinant inbred mouse strain. Lung homogenate 72 hours post H5N
## GSM1579278_jac032-430v2.CEL BXD97 Recombinant inbred mouse strain. Lung homogenate 72 hours post H5N
## GSM1579277_jac031-430v2.CEL BXD97 Recombinant inbred mouse strain. Lung homogenate 72 hours post H5N
## GSM1579276_jac030-430v2.CEL BXD97 Recombinant inbred mouse strain. Lung homogenate 72 hours post H5N
##                             Characteristics..age. Characteristics..organism.
## GSM1579281_jac040-430v2.CEL             6-8wk old               Mus musculus
## GSM1579280_jac039-430v2.CEL             6-8wk old               Mus musculus
## GSM1579279_jac038-430v2.CEL             6-8wk old               Mus musculus
## GSM1579278_jac032-430v2.CEL             6-8wk old               Mus musculus
## GSM1579277_jac031-430v2.CEL             6-8wk old               Mus musculus
## GSM1579276_jac030-430v2.CEL             6-8wk old               Mus musculus
##                             Term.Source.REF
## GSM1579281_jac040-430v2.CEL             EFO
## GSM1579280_jac039-430v2.CEL             EFO
## GSM1579279_jac038-430v2.CEL             EFO
## GSM1579278_jac032-430v2.CEL             EFO
## GSM1579277_jac031-430v2.CEL             EFO
## GSM1579276_jac030-430v2.CEL             EFO
##                                                Term.Accession.Number
## GSM1579281_jac040-430v2.CEL http://purl.obolibrary.org/obo/NCBITaxon_10090
## GSM1579280_jac039-430v2.CEL http://purl.obolibrary.org/obo/NCBITaxon_10090
## GSM1579279_jac038-430v2.CEL http://purl.obolibrary.org/obo/NCBITaxon_10090
## GSM1579278_jac032-430v2.CEL http://purl.obolibrary.org/obo/NCBITaxon_10090
## GSM1579277_jac031-430v2.CEL http://purl.obolibrary.org/obo/NCBITaxon_10090
## GSM1579276_jac030-430v2.CEL http://purl.obolibrary.org/obo/NCBITaxon_10090
##                             Characteristics..sex. Term.Source.REF.1
## GSM1579281_jac040-430v2.CEL                female               EFO
```

```
## GSM1579280_jac039-430v2.CEL                female                EFO
## GSM1579279_jac038-430v2.CEL                female                EFO
## GSM1579278_jac032-430v2.CEL                female                EFO
## GSM1579277_jac031-430v2.CEL                female                EFO
## GSM1579276_jac030-430v2.CEL                female                EFO
##                             Term.Accession.Number.1 Characteristics..strain.
## GSM1579281_jac040-430v2.CEL            EFO_0001265                    BXD98
## GSM1579280_jac039-430v2.CEL            EFO_0001265                    BXD98
## GSM1579279_jac038-430v2.CEL            EFO_0001265                    BXD98
## GSM1579278_jac032-430v2.CEL            EFO_0001265                    BXD97
## GSM1579277_jac031-430v2.CEL            EFO_0001265                    BXD97
## GSM1579276_jac030-430v2.CEL            EFO_0001265                    BXD97
##                             Term.Source.REF.2 Term.Accession.Number.2
## GSM1579281_jac040-430v2.CEL
## GSM1579280_jac039-430v2.CEL
## GSM1579279_jac038-430v2.CEL
## GSM1579278_jac032-430v2.CEL
## GSM1579277_jac031-430v2.CEL
## GSM1579276_jac030-430v2.CEL
##                             Protocol.REF Term.Source.REF.3 Protocol.REF.1
## GSM1579281_jac040-430v2.CEL P-GSE64750-2      ArrayExpress   P-GSE64750-3
## GSM1579280_jac039-430v2.CEL P-GSE64750-2      ArrayExpress   P-GSE64750-3
## GSM1579279_jac038-430v2.CEL P-GSE64750-2      ArrayExpress   P-GSE64750-3
## GSM1579278_jac032-430v2.CEL P-GSE64750-2      ArrayExpress   P-GSE64750-3
## GSM1579277_jac031-430v2.CEL P-GSE64750-2      ArrayExpress   P-GSE64750-3
## GSM1579276_jac030-430v2.CEL P-GSE64750-2      ArrayExpress   P-GSE64750-3
##                             Term.Source.REF.4         Extract.Name
## GSM1579281_jac040-430v2.CEL      ArrayExpress GSM1579281 extract 1
## GSM1579280_jac039-430v2.CEL      ArrayExpress GSM1579280 extract 1
## GSM1579279_jac038-430v2.CEL      ArrayExpress GSM1579279 extract 1
## GSM1579278_jac032-430v2.CEL      ArrayExpress GSM1579278 extract 1
## GSM1579277_jac031-430v2.CEL      ArrayExpress GSM1579277 extract 1
## GSM1579276_jac030-430v2.CEL      ArrayExpress GSM1579276 extract 1
##                             Material.Type Protocol.REF.2 Term.Source.REF.5
## GSM1579281_jac040-430v2.CEL     total RNA   P-GSE64750-4      ArrayExpress
## GSM1579280_jac039-430v2.CEL     total RNA   P-GSE64750-4      ArrayExpress
## GSM1579279_jac038-430v2.CEL     total RNA   P-GSE64750-4      ArrayExpress
## GSM1579278_jac032-430v2.CEL     total RNA   P-GSE64750-4      ArrayExpress
## GSM1579277_jac031-430v2.CEL     total RNA   P-GSE64750-4      ArrayExpress
## GSM1579276_jac030-430v2.CEL     total RNA   P-GSE64750-4      ArrayExpress
##                             Labeled.Extract.Name  Label Protocol.REF.3
## GSM1579281_jac040-430v2.CEL      GSM1579281 LE 1 biotin   P-GSE64750-5
## GSM1579280_jac039-430v2.CEL      GSM1579280 LE 1 biotin   P-GSE64750-5
## GSM1579279_jac038-430v2.CEL      GSM1579279 LE 1 biotin   P-GSE64750-5
## GSM1579278_jac032-430v2.CEL      GSM1579278 LE 1 biotin   P-GSE64750-5
## GSM1579277_jac031-430v2.CEL      GSM1579277 LE 1 biotin   P-GSE64750-5
## GSM1579276_jac030-430v2.CEL      GSM1579276 LE 1 biotin   P-GSE64750-5
##                             Term.Source.REF.6 Assay.Name Array.Design.REF
## GSM1579281_jac040-430v2.CEL      ArrayExpress GSM1579281        A-AFFY-45
## GSM1579280_jac039-430v2.CEL      ArrayExpress GSM1579280        A-AFFY-45
## GSM1579279_jac038-430v2.CEL      ArrayExpress GSM1579279        A-AFFY-45
## GSM1579278_jac032-430v2.CEL      ArrayExpress GSM1579278        A-AFFY-45
## GSM1579277_jac031-430v2.CEL      ArrayExpress GSM1579277        A-AFFY-45
## GSM1579276_jac030-430v2.CEL      ArrayExpress GSM1579276        A-AFFY-45
```

```
##                              Term.Source.REF.7 Technology.Type Protocol.REF.4
## GSM1579281_jac040-430v2.CEL      ArrayExpress     array assay   P-GSE64750-6
## GSM1579280_jac039-430v2.CEL      ArrayExpress     array assay   P-GSE64750-6
## GSM1579279_jac038-430v2.CEL      ArrayExpress     array assay   P-GSE64750-6
## GSM1579278_jac032-430v2.CEL      ArrayExpress     array assay   P-GSE64750-6
## GSM1579277_jac031-430v2.CEL      ArrayExpress     array assay   P-GSE64750-6
## GSM1579276_jac030-430v2.CEL      ArrayExpress     array assay   P-GSE64750-6
##                              Term.Source.REF.8             Array.Data.File
## GSM1579281_jac040-430v2.CEL      ArrayExpress GSM1579281_jac040-430v2.CEL
## GSM1579280_jac039-430v2.CEL      ArrayExpress GSM1579280_jac039-430v2.CEL
## GSM1579279_jac038-430v2.CEL      ArrayExpress GSM1579279_jac038-430v2.CEL
## GSM1579278_jac032-430v2.CEL      ArrayExpress GSM1579278_jac032-430v2.CEL
## GSM1579277_jac031-430v2.CEL      ArrayExpress GSM1579277_jac031-430v2.CEL
## GSM1579276_jac030-430v2.CEL      ArrayExpress GSM1579276_jac030-430v2.CEL
##                                                                                                                                Co
## GSM1579281_jac040-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579280_jac039-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579279_jac038-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579278_jac032-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579277_jac031-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579276_jac030-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
##                              Protocol.REF.5 Term.Source.REF.9
## GSM1579281_jac040-430v2.CEL   P-GSE64750-1      ArrayExpress
## GSM1579280_jac039-430v2.CEL   P-GSE64750-1      ArrayExpress
## GSM1579279_jac038-430v2.CEL   P-GSE64750-1      ArrayExpress
## GSM1579278_jac032-430v2.CEL   P-GSE64750-1      ArrayExpress
## GSM1579277_jac031-430v2.CEL   P-GSE64750-1      ArrayExpress
## GSM1579276_jac030-430v2.CEL   P-GSE64750-1      ArrayExpress
##                                                Normalization.Name
## GSM1579281_jac040-430v2.CEL GSM1579281_sample_table.txt norm
## GSM1579280_jac039-430v2.CEL GSM1579280_sample_table.txt norm
## GSM1579279_jac038-430v2.CEL GSM1579279_sample_table.txt norm
## GSM1579278_jac032-430v2.CEL GSM1579278_sample_table.txt norm
## GSM1579277_jac031-430v2.CEL GSM1579277_sample_table.txt norm
## GSM1579276_jac030-430v2.CEL GSM1579276_sample_table.txt norm
##                                  Derived.Array.Data.File
## GSM1579281_jac040-430v2.CEL GSM1579281_sample_table.txt
## GSM1579280_jac039-430v2.CEL GSM1579280_sample_table.txt
## GSM1579279_jac038-430v2.CEL GSM1579279_sample_table.txt
## GSM1579278_jac032-430v2.CEL GSM1579278_sample_table.txt
## GSM1579277_jac031-430v2.CEL GSM1579277_sample_table.txt
## GSM1579276_jac030-430v2.CEL GSM1579276_sample_table.txt
##                                                                                                                                Comm
## GSM1579281_jac040-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579280_jac039-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579279_jac038-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579278_jac032-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579277_jac031-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
## GSM1579276_jac030-430v2.CEL ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/GEOD/E-GEOD-
##                              FactorValue..strain. Term.Source.REF.10
## GSM1579281_jac040-430v2.CEL                 BXD98
## GSM1579280_jac039-430v2.CEL                 BXD98
## GSM1579279_jac038-430v2.CEL                 BXD98
## GSM1579278_jac032-430v2.CEL                 BXD97
```

```
## GSM1579277_jac031-430v2.CEL                BXD97
## GSM1579276_jac030-430v2.CEL                BXD97
##                             Term.Accession.Number.3
## GSM1579281_jac040-430v2.CEL
## GSM1579280_jac039-430v2.CEL
## GSM1579279_jac038-430v2.CEL
## GSM1579278_jac032-430v2.CEL
## GSM1579277_jac031-430v2.CEL
## GSM1579276_jac030-430v2.CEL
```

```
filter2 <- colnames(data.raw_2)[data.raw_2@phenoData@data$index <= 16]
filter2
```

```
##  [1] "GSM1579245_jac013-430v2.CEL" "GSM1579246_jac021-430v2.CEL"
##  [3] "GSM1579247_jac044-430v2.CEL" "GSM1579248_jac045-430v2.CEL"
##  [5] "GSM1579249_jac046-430v2.CEL" "GSM1579250_jac007-430v2.CEL"
##  [7] "GSM1579251_jac008-430v2.CEL" "GSM1579252_jac036-430v2.CEL"
##  [9] "GSM1579253_jac047-430v2.CEL" "GSM1579254_jac014-430v2.CEL"
## [11] "GSM1579255_jac024-430v2.CEL" "GSM1579256_jac025-430v2.CEL"
## [13] "GSM1579257_jac011-430v2.CEL" "GSM1579258_jac012-430v2.CEL"
## [15] "GSM1579259_jac037-430v2.CEL" "GSM1579260_jac048-430v2.CEL"
```

```
filtered2 <- data.raw_2[,filter2]
filtered2
```

```
## ExpressionFeatureSet (storageMode: lockedEnvironment)
## assayData: 1004004 features, 16 samples
##   element names: exprs
## protocolData
##   rowNames: GSM1579245_jac013-430v2.CEL GSM1579246_jac021-430v2.CEL ...
##     GSM1579260_jac048-430v2.CEL (16 total)
##   varLabels: exprs dates
##   varMetadata: labelDescription channel
## phenoData
##   rowNames: GSM1579245_jac013-430v2.CEL GSM1579246_jac021-430v2.CEL ...
##     GSM1579260_jac048-430v2.CEL (16 total)
##   varLabels: index
##   varMetadata: labelDescription channel
## featureData: none
## experimentData: use 'experimentData(object)'
## Annotation: pd.mouse430.2
```

```
dim(exprs(filtered))  #1004004 features       9 samples
```

```
## [1] 1102500       6
```

```
head(exprs(filtered2))
```

```
##   GSM1579245_jac013-430v2.CEL GSM1579246_jac021-430v2.CEL
## 1                          62                         191
## 2                        5707                       16272
```

```
## 3                          69                          265
## 4                        5913                        16107
## 5                          57                          143
## 6                          54                          143
##   GSM1579247_jac044-430v2.CEL GSM1579248_jac045-430v2.CEL
## 1                         198                         134
## 2                        7485                        5697
## 3                         200                         144
## 4                        7664                        6184
## 5                         154                         121
## 6                         162                         128
##   GSM1579249_jac046-430v2.CEL GSM1579250_jac007-430v2.CEL
## 1                         136                         123
## 2                        6036                        7786
## 3                         134                         162
## 4                        6147                        7832
## 5                         126                         111
## 6                         113                         105
##   GSM1579251_jac008-430v2.CEL GSM1579252_jac036-430v2.CEL
## 1                         139                          80
## 2                        8743                        6310
## 3                         154                         102
## 4                        8940                        6335
## 5                         102                          70
## 6                          96                          88
##   GSM1579253_jac047-430v2.CEL GSM1579254_jac014-430v2.CEL
## 1                          92                         105
## 2                        4988                        8341
## 3                          87                         159
## 4                        5275                        8721
## 5                          87                         109
## 6                          79                          96
##   GSM1579255_jac024-430v2.CEL GSM1579256_jac025-430v2.CEL
## 1                         125                         153
## 2                        9656                        8775
## 3                         200                         180
## 4                       10228                        8859
## 5                          94                         101
## 6                         118                         121
##   GSM1579257_jac011-430v2.CEL GSM1579258_jac012-430v2.CEL
## 1                         182                         130
## 2                       13154                        9498
## 3                         218                         195
## 4                       13689                        9705
## 5                         104                         108
## 6                         132                         128
##   GSM1579259_jac037-430v2.CEL GSM1579260_jac048-430v2.CEL
## 1                         176                          92
## 2                       15363                        4973
## 3                         251                          72
## 4                       15737                        5058
## 5                         142                          72
## 6                         173                          63
```

```
#arrayQualityMetrics(filtered2,outdir="./raw2",force=T)
#arrayQualityMetrics(filtered2,outdir="./rawlog2",force=T,do.logtransform=T)

miceRMA <- oligo::rma(filtered2, background=T)
```

```
## Background correcting
## Normalizing
## Calculating Expression
```

```
head(miceRMA)
```

```
## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 6 features, 16 samples
##   element names: exprs
## protocolData
##   rowNames: GSM1579245_jac013-430v2.CEL GSM1579246_jac021-430v2.CEL ...
##     GSM1579260_jac048-430v2.CEL (16 total)
##   varLabels: exprs dates
##   varMetadata: labelDescription channel
## phenoData
##   rowNames: GSM1579245_jac013-430v2.CEL GSM1579246_jac021-430v2.CEL ...
##     GSM1579260_jac048-430v2.CEL (16 total)
##   varLabels: index
##   varMetadata: labelDescription channel
## featureData: none
## experimentData: use 'experimentData(object)'
## Annotation: pd.mouse430.2
```

```
#arrayQualityMetrics(miceRMA, outdir="./rma2", force=TRUE)
```

```
## Differential expression analysis with RMA preprocessed data
####################

## Additional preprocessing
samples <- c(replicate(5, "DBA/2J control"), replicate(4, "DBA/2J infected"), replicate(3, "C57/BL6J co
samples
```

```
##  [1] "DBA/2J control"    "DBA/2J control"    "DBA/2J control"
##  [4] "DBA/2J control"    "DBA/2J control"    "DBA/2J infected"
##  [7] "DBA/2J infected"   "DBA/2J infected"   "DBA/2J infected"
## [10] "C57/BL6J control"  "C57/BL6J control"  "C57/BL6J control"
## [13] "C57/BL6J infected" "C57/BL6J infected" "C57/BL6J infected"
## [16] "C57/BL6J infected"
```

```
condition <- c(replicate(5, "control"), replicate(4, "infected"), replicate(3, "control"), replicate(4,

pData(miceRMA)[,2] <- condition
pData(miceRMA)[,3] <- c(replicate(9, "DBA/2J"), replicate(7, "C57/BL6J"))
pData(miceRMA)[,4] <- samples

colnames(pData(miceRMA)) <- c("index", "condition", "strain", "samples")
pData(miceRMA)
```

```
##                              index condition   strain           samples
## GSM1579245_jac013-430v2.CEL     1   control   DBA/2J    DBA/2J control
## GSM1579246_jac021-430v2.CEL     2   control   DBA/2J    DBA/2J control
## GSM1579247_jac044-430v2.CEL     3   control   DBA/2J    DBA/2J control
## GSM1579248_jac045-430v2.CEL     4   control   DBA/2J    DBA/2J control
## GSM1579249_jac046-430v2.CEL     5   control   DBA/2J    DBA/2J control
## GSM1579250_jac007-430v2.CEL     6  infected   DBA/2J   DBA/2J infected
## GSM1579251_jac008-430v2.CEL     7  infected   DBA/2J   DBA/2J infected
## GSM1579252_jac036-430v2.CEL     8  infected   DBA/2J   DBA/2J infected
## GSM1579253_jac047-430v2.CEL     9  infected   DBA/2J   DBA/2J infected
## GSM1579254_jac014-430v2.CEL    10   control C57/BL6J  C57/BL6J control
## GSM1579255_jac024-430v2.CEL    11   control C57/BL6J  C57/BL6J control
## GSM1579256_jac025-430v2.CEL    12   control C57/BL6J  C57/BL6J control
## GSM1579257_jac011-430v2.CEL    13  infected C57/BL6J C57/BL6J infected
## GSM1579258_jac012-430v2.CEL    14  infected C57/BL6J C57/BL6J infected
## GSM1579259_jac037-430v2.CEL    15  infected C57/BL6J C57/BL6J infected
## GSM1579260_jac048-430v2.CEL    16  infected C57/BL6J C57/BL6J infected
```

The variability of the strain is encompassed in the model but you do not test for it

```
condition <- factor(pData(miceRMA)[,2])
strain <- factor(pData(miceRMA)[,3])
condition
```

```
##  [1] control  control  control  control  control  infected infected infected
##  [9] infected control  control  control  infected infected infected infected
## Levels: control infected
```

```
strain
```

```
##  [1] DBA/2J   DBA/2J   DBA/2J   DBA/2J   DBA/2J   DBA/2J   DBA/2J   DBA/2J
##  [9] DBA/2J   C57/BL6J C57/BL6J C57/BL6J C57/BL6J C57/BL6J C57/BL6J C57/BL6J
## Levels: C57/BL6J DBA/2J
```

```
design <- model.matrix(~0+condition*strain)
colnames(design)<-c("Control","Infected", "strain","interaction")
# for strain, 1 represents DBA/2J, 0 represents c57/BL6
design
```

```
##    Control Infected strain interaction
## 1        1        0      1           0
## 2        1        0      1           0
## 3        1        0      1           0
## 4        1        0      1           0
## 5        1        0      1           0
## 6        0        1      1           1
## 7        0        1      1           1
## 8        0        1      1           1
## 9        0        1      1           1
## 10       1        0      0           0
## 11       1        0      0           0
## 12       1        0      0           0
```

```
## 13       0        1      0           0
## 14       0        1      0           0
## 15       0        1      0           0
## 16       0        1      0           0
## attr(,"assign")
## [1] 1 1 2 3
## attr(,"contrasts")
## attr(,"contrasts")$condition
## [1] "contr.treatment"
##
## attr(,"contrasts")$strain
## [1] "contr.treatment"
```

```
fit_m2 <- lmFit(miceRMA, design)
cont.matrix <- makeContrasts(InfectedvsControl="Infected-Control", levels=design)
cont.matrix
```

```
##              Contrasts
## Levels        InfectedvsControl
##   Control                    -1
##   Infected                    1
##   strain                      0
##   interaction                 0
```

```
fit2_m2 <- contrasts.fit(fit_m2,cont.matrix)
fit2_m2 <- eBayes(fit2_m2)
fit2_m2
```

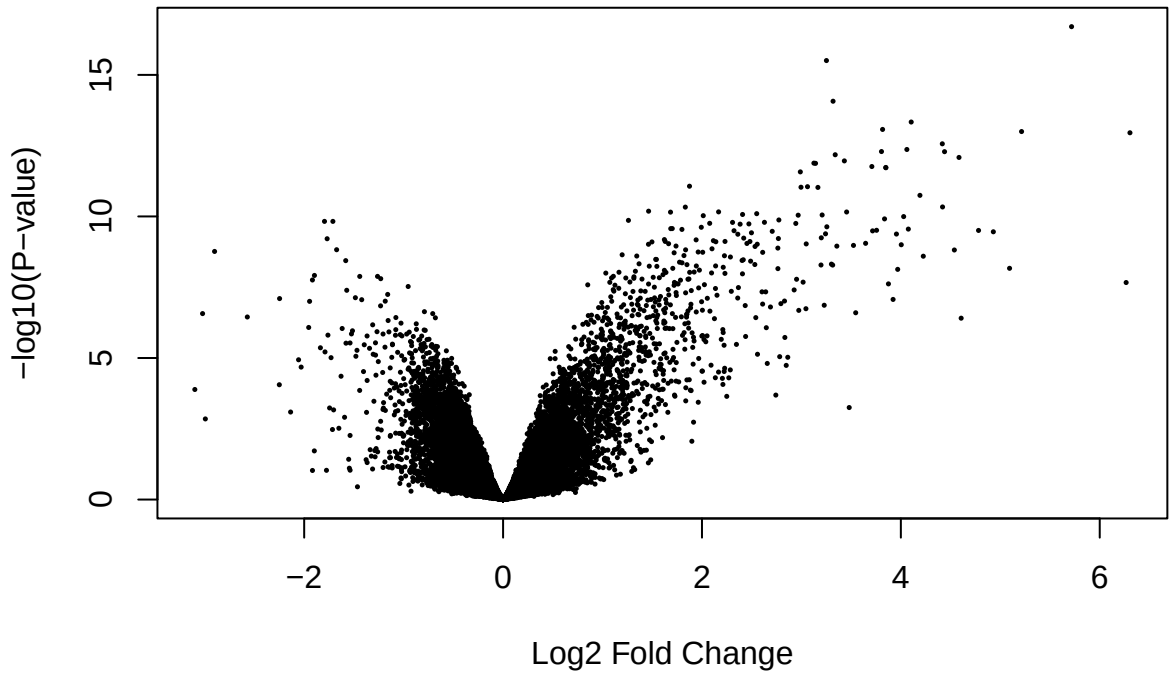
```
## An object of class "MArrayLM"
## $coefficients
##               Contrasts
##                InfectedvsControl
##   1415670_at         0.295615220
##   1415671_at         0.108222965
##   1415672_at        -0.001215109
##   1415673_at        -0.023467868
##   1415674_a_at       0.173622489
## 45096 more rows ...
##
## $rank
## [1] 4
##
## $assign
## [1] 1 1 2 3
##
## $qr
## $qr
##      Control  Infected     strain interaction
## 1 -2.8284271  0.000000 -1.7677670   0.0000000
## 2  0.3535534 -2.828427 -1.4142136  -1.4142136
## 3  0.3535534  0.000000 -1.9685020  -1.0160010
## 4  0.3535534  0.000000  0.1407408   0.9837388
## 5  0.3535534  0.000000  0.1407408   0.1274229
```

```
## 11 more rows ...
##
## $qraux
## [1] 1.353553 1.000000 1.140741 1.127423
##
## $pivot
## [1] 1 2 3 4
##
## $tol
## [1] 1e-07
##
## $rank
## [1] 4
##
##
## $df.residual
## [1] 12 12 12 12 12
## 45096 more elements ...
##
## $sigma
##   1415670_at   1415671_at   1415672_at   1415673_at 1415674_a_at
##    0.3165328    0.2033128    0.1071823    0.3166509    0.1363164
## 45096 more elements ...
##
## $cov.coefficients
##                     Contrasts
## Contrasts            InfectedvsControl
##   InfectedvsControl         0.5833333
##
## $stdev.unscaled
##               Contrasts
##                InfectedvsControl
##   1415670_at           0.7637626
##   1415671_at           0.7637626
##   1415672_at           0.7637626
##   1415673_at           0.7637626
##   1415674_a_at         0.7637626
## 45096 more rows ...
##
## $pivot
## [1] 1 2 3 4
##
## $Amean
##   1415670_at   1415671_at   1415672_at   1415673_at 1415674_a_at
##     8.995360    10.365534    10.828212     7.422040     9.235163
## 45096 more elements ...
##
## $method
## [1] "ls"
##
## $design
##   Control Infected strain interaction
## 1       1        0      1           0
## 2       1        0      1           0
```

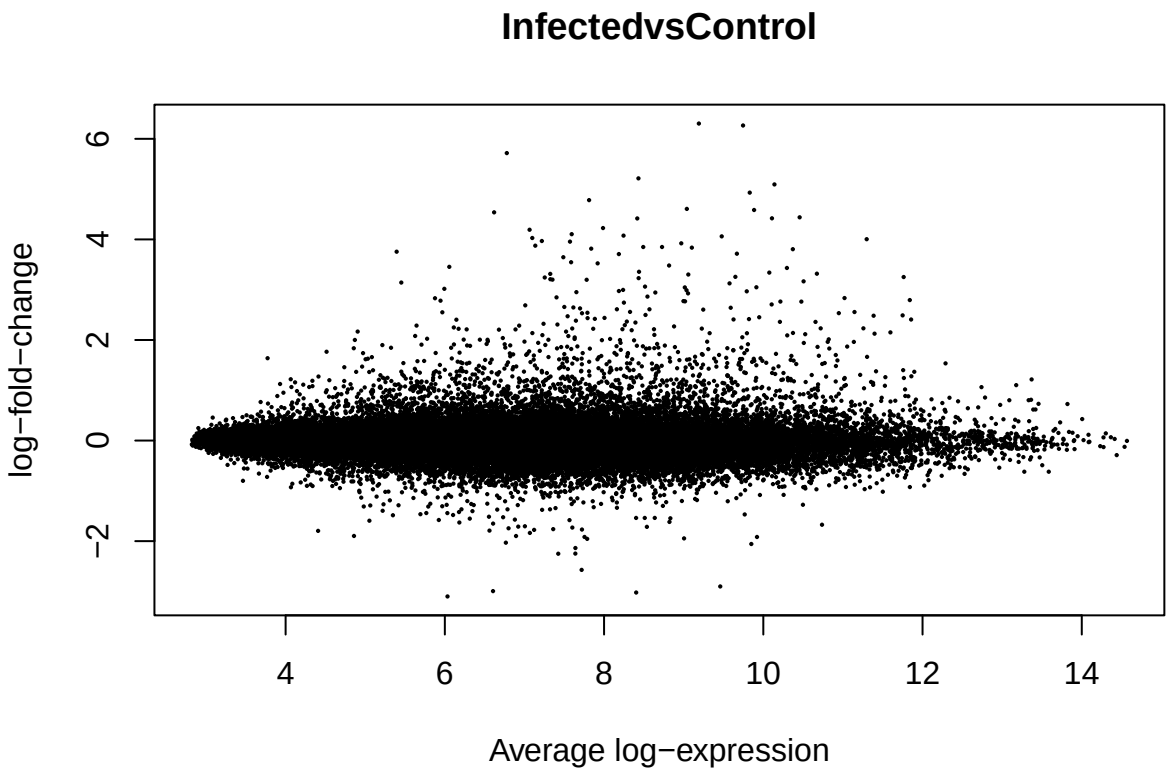
```
## 3       1        0      1           0
## 4       1        0      1           0
## 5       1        0      1           0
## 11 more rows ...
##
## $contrasts
##             Contrasts
## Levels        InfectedvsControl
##   Control                    -1
##   Infected                    1
##   strain                      0
##   interaction                 0
##
## $df.prior
## [1] 3.078919
##
## $s2.prior
## [1] 0.03390424
##
## $var.prior
## [1] 61.79253
##
## $proportion
## [1] 0.01
##
## $s2.post
##   1415670_at   1415671_at   1415672_at   1415673_at 1415674_a_at
##   0.08665770   0.03981860   0.01606514   0.08671723   0.02171072
## 45096 more elements ...
##
## $t
##               Contrasts
##                InfectedvsControl
##   1415670_at          1.31481549
##   1415671_at          0.71009763
##   1415672_at         -0.01255205
##   1415673_at         -0.10434281
##   1415674_a_at        1.54280288
## 45096 more rows ...
##
## $df.total
## [1] 15.07892 15.07892 15.07892 15.07892 15.07892
## 45096 more elements ...
##
## $p.value
##               Contrasts
##                InfectedvsControl
##   1415670_at           0.2082185
##   1415671_at           0.4884808
##   1415672_at           0.9901497
##   1415673_at           0.9182725
##   1415674_a_at         0.1436020
## 45096 more rows ...
##
```

```
## $lods
##               Contrasts
##                InfectedvsControl
##   1415670_at           -6.067244
##   1415671_at           -6.669279
##   1415672_at           -6.931124
##   1415673_at           -6.925459
##   1415674_a_at         -5.764749
## 45096 more rows ...
##
## $F
## [1] 1.7287397757 0.5042386432 0.0001575539 0.0108874228 2.3802407312
## 45096 more elements ...
##
## $F.p.value
## [1] 0.2082185 0.4884808 0.9901497 0.9182725 0.1436020
## 45096 more elements ...
```

```
volcanoplot(fit2_m2)
```



```
limma::plotMA(fit2_m2)
```




```
# DE results
LIMMAout2 <- topTable(fit2_m2,adjust="BH",number=nrow(exprs(miceRMA)))
#head(LIMMAout)

## Check intensity values for top results
head(exprs(miceRMA)[rownames(exprs(miceRMA))%in%rownames(head(LIMMAout2)),])
```

```
##             GSM1579245_jac013-430v2.CEL GSM1579246_jac021-430v2.CEL
## 1418293_at                     8.850220                    8.697405
## 1419697_at                     4.873087                    5.196294
## 1424518_at                     5.610642                    5.255470
## 1449025_at                     9.824438                    9.841855
## 1450297_at                     3.450449                    3.409701
## 1451905_a_at                   5.271445                    5.483409
##             GSM1579247_jac044-430v2.CEL GSM1579248_jac045-430v2.CEL
## 1418293_at                     8.628802                    8.583339
## 1419697_at                     4.944273                    4.957930
## 1424518_at                     5.033368                    5.491428
## 1449025_at                     9.930787                    9.733168
## 1450297_at                     3.628502                    3.614535
## 1451905_a_at                   5.814011                    5.916976
##             GSM1579249_jac046-430v2.CEL GSM1579250_jac007-430v2.CEL
## 1418293_at                     8.979707                   13.280513
```

```
## 1419697_at                      5.156156                     12.484048
## 1424518_at                      5.391127                      9.926284
## 1449025_at                     10.059654                     13.751370
## 1450297_at                      3.661825                     10.544846
## 1451905_a_at                    6.290288                     12.039855
##              GSM1579251_jac008-430v2.CEL GSM1579252_jac036-430v2.CEL
## 1418293_at                     13.17569                     13.17675
## 1419697_at                     12.58070                     12.54747
## 1424518_at                     10.07358                     10.38063
## 1449025_at                     13.69643                     13.85968
## 1450297_at                     10.77543                     10.84424
## 1451905_a_at                   11.65031                     12.11285
##              GSM1579253_jac047-430v2.CEL GSM1579254_jac014-430v2.CEL
## 1418293_at                     13.07904                     8.738460
## 1419697_at                     12.59532                     4.815025
## 1424518_at                     10.27056                     5.208682
## 1449025_at                     13.77391                    10.119994
## 1450297_at                     10.40172                     3.770888
## 1451905_a_at                   11.95163                     5.430919
##              GSM1579255_jac024-430v2.CEL GSM1579256_jac025-430v2.CEL
## 1418293_at                     8.723118                     8.699255
## 1419697_at                     5.156243                     4.948877
## 1424518_at                     5.235854                     5.688553
## 1449025_at                    10.063244                    10.129423
## 1450297_at                     3.496160                     3.556766
## 1451905_a_at                   5.635768                     5.014256
##              GSM1579257_jac011-430v2.CEL GSM1579258_jac012-430v2.CEL
## 1418293_at                    12.021105                    12.276407
## 1419697_at                     8.623987                     9.287277
## 1424518_at                     9.305259                     9.485391
## 1449025_at                    13.289408                    13.458878
## 1450297_at                     9.421028                     9.491732
## 1451905_a_at                  10.501785                    10.709535
##              GSM1579259_jac037-430v2.CEL GSM1579260_jac048-430v2.CEL
## 1418293_at                    11.864963                    11.991672
## 1419697_at                     8.614776                     8.632264
## 1424518_at                     9.530186                     9.606000
## 1449025_at                    13.312026                    13.363678
## 1450297_at                     9.073683                     9.311915
## 1451905_a_at                  10.413056                    10.672276
```

```
#mean expression of control/DBA2J
rowMeans(exprs(miceRMA)[rownames(exprs(miceRMA))%in%rownames(head(LIMMAout2)),1:5])
```

```
##   1418293_at   1419697_at   1424518_at   1449025_at   1450297_at 1451905_a_at
##     8.747894     5.025548     5.356407     9.877981     3.553002     5.755226
```

```
#mean expression of infected/DBA2J
rowMeans(exprs(miceRMA)[rownames(exprs(miceRMA))%in%rownames(head(LIMMAout2)),6:9])
```

```
##   1418293_at   1419697_at   1424518_at   1449025_at   1450297_at 1451905_a_at
##     13.17800     12.55189     10.16277     13.77035     10.64156     11.93866
```

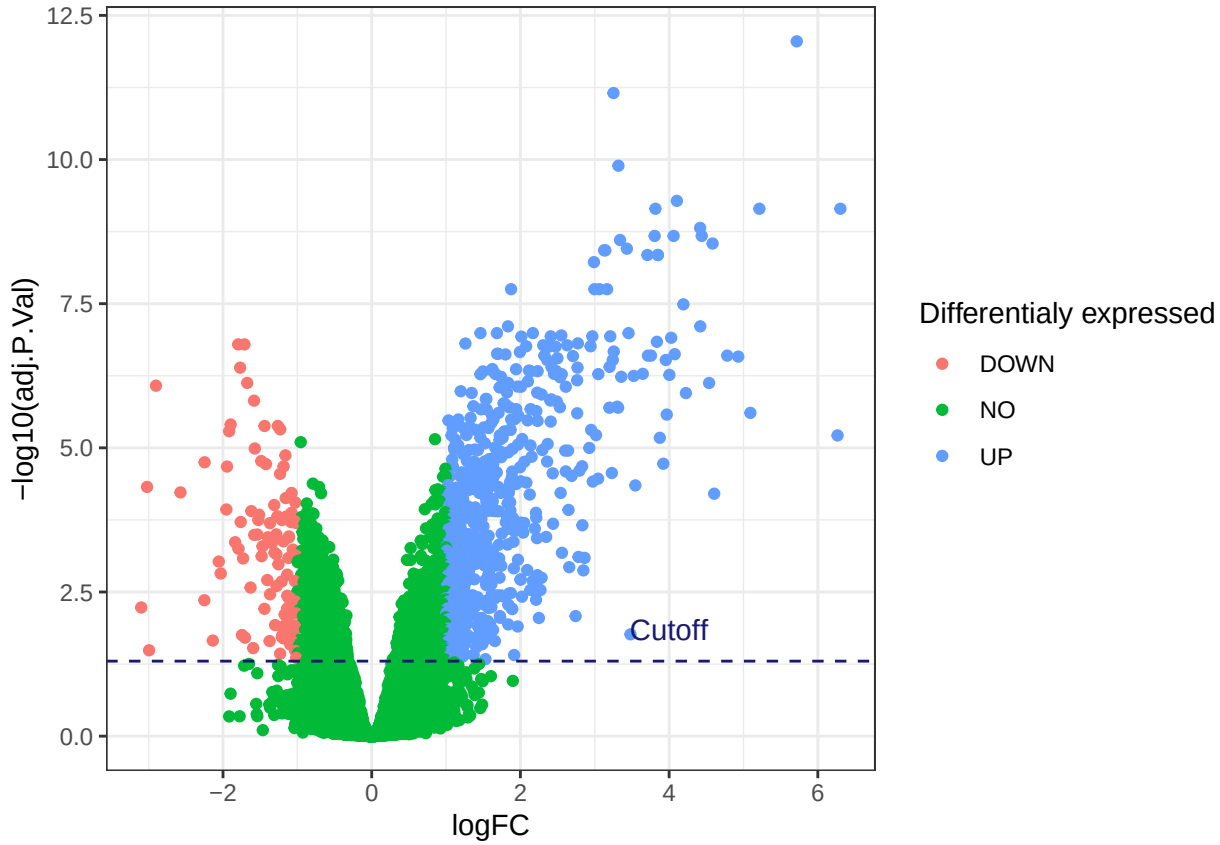
```r
#mean expression of control/C57BL6
rowMeans(exprs(miceRMA)[rownames(exprs(miceRMA))%in%rownames(head(LIMMAout2)),10:12])
```

```
##   1418293_at   1419697_at   1424518_at   1449025_at   1450297_at 1451905_a_at
##     8.720277     4.973382     5.377696    10.104220     3.607938     5.360314
```

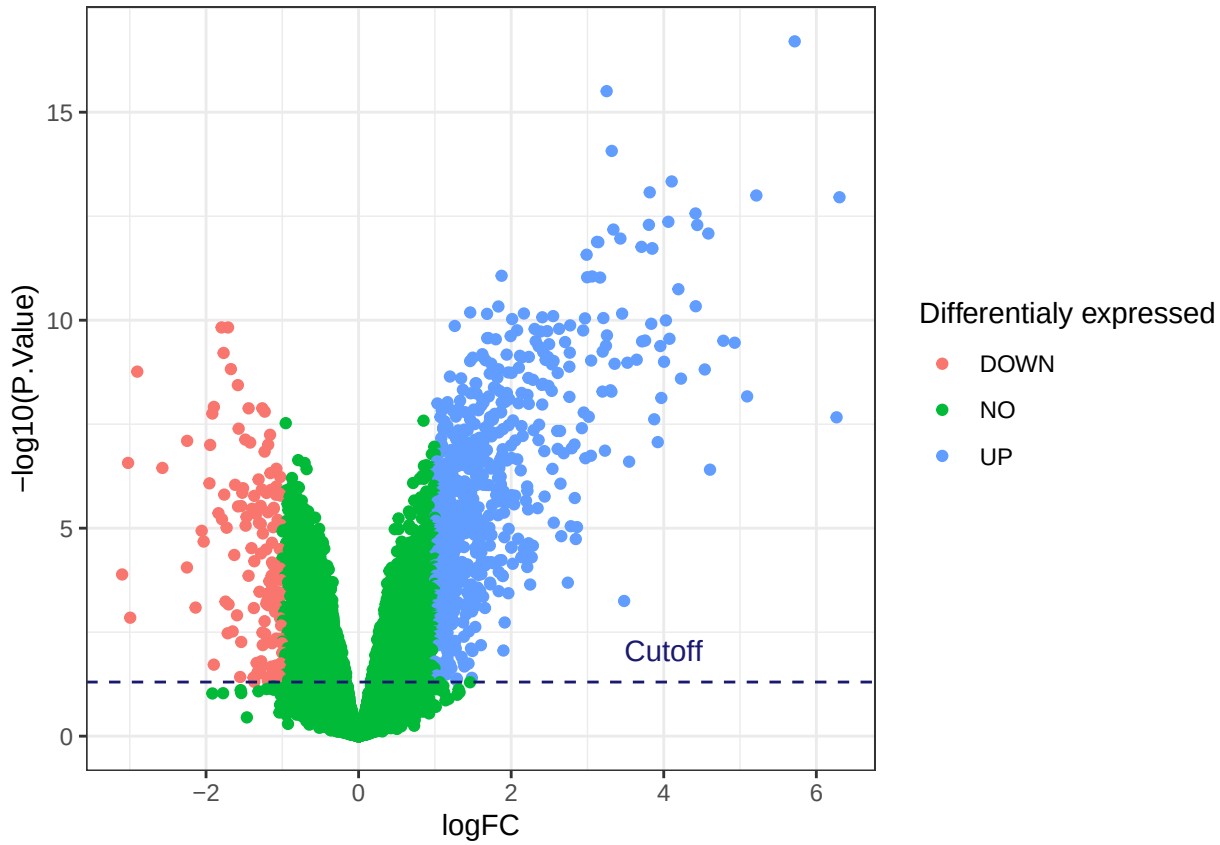
```r
#mean expression of infected/C57BL6
rowMeans(exprs(miceRMA)[rownames(exprs(miceRMA))%in%rownames(head(LIMMAout2)),13:16])
```

```
##   1418293_at   1419697_at   1424518_at   1449025_at   1450297_at 1451905_a_at
##    12.038537     8.789576     9.481709    13.355997     9.324589    10.574163
```

```r
#Adjustments on p values using Benjamini-Hochberg
LIMMAout2$diffexpressed <- "NO"
LIMMAout2$diffexpressed[LIMMAout2$logFC > 1 & LIMMAout2$adj.P.Val < 0.05] <- "UP"
LIMMAout2$diffexpressed[LIMMAout2$logFC < -1 & LIMMAout2$adj.P.Val < 0.05] <- "DOWN"

#No adjustments on pvalues
LIMMAout2$diffexpressed_no_BH <- "NO"
LIMMAout2$diffexpressed_no_BH[LIMMAout2$logFC > 1 & LIMMAout2$P.Value < 0.05] <- "UP"
LIMMAout2$diffexpressed_no_BH[LIMMAout2$logFC < -1 & LIMMAout2$P.Value < 0.05] <- "DOWN"

#jpeg("microarray2_volcanoplot.jpg")
ggplot(data = LIMMAout2, aes(x= logFC, y = -log10(adj.P.Val), colour = diffexpressed)) +
 geom_point()+
 theme_bw()+
  geom_hline(yintercept = -log10(0.05), linetype="dashed", color="midnightblue")+
  annotate("text", min(4), 1.3, vjust = -1, label = "Cutoff", color="midnightblue")+
 #ggtitle("Differentiall (unadjusted P-value)") +
 labs(colour = "Differentialy expressed")
```




```
 #theme(plot.title = element_text(hjust = 0.5, face = "bold.italic")dev.off()

ggplot(data = LIMMAout2, aes(x= logFC, y = -log10(P.Value), colour = diffexpressed_no_BH)) +
 geom_point()+
 theme_bw()+
  geom_hline(yintercept = -log10(0.05), linetype="dashed", color="midnightblue")+
  annotate("text", min(4), 1.3, vjust = -1, label = "Cutoff", color="midnightblue")+
 #ggtitle("Differentiall (unadjusted P-value)") +
 labs(colour = "Differentialy expressed")
```




```
length(which(LIMMAout2$diffexpressed=="UP"))    #641 upregulated
```

```
## [1] 641
```

```
length(which(LIMMAout2$diffexpressed=="DOWN"))  #100 downregulated
```

```
## [1] 100
```

```
length(which(LIMMAout2$diffexpressed_no_BH=="UP"))  #701 upregulated
```

```
## [1] 701
```

```
length(which(LIMMAout2$diffexpressed_no_BH=="DOWN")) #136 downregulated
```

```
## [1] 136
```

```
## Load annotation and sort alphabetically on probe name
setwd("../Datasets/Microarray2/")
annotation_MA2 <- read.table("A-AFFY-45.adf.txt",header=T,sep="\t",skip=17,fill=T)
print(head(annotation_MA2))
```

```
##   Composite.Element.Name Composite.Element.Database.Entry.interpro.
## 1         AFFX-BioB-5_at                                   IPR007197
## 2         AFFX-BioB-M_at                                   IPR007197
## 3         AFFX-BioB-3_at                                   IPR007197
## 4         AFFX-BioC-5_at
## 5         AFFX-BioC-3_at
## 6        AFFX-BioDn-5_at                                   IPR002586
##   Composite.Element.Database.Entry.embl.
## 1                            AFFX-BioB-5
## 2                            AFFX-BioB-M
## 3                            AFFX-BioB-3
## 4                            AFFX-BioC-5
## 5                            AFFX-BioC-3
## 6                           AFFX-BioDn-5
##   Composite.Element.Database.Entry.affymetrix_netaffx.
## 1                                      AFFX-BioB-5_at
## 2                                      AFFX-BioB-M_at
## 3                                      AFFX-BioB-3_at
## 4                                      AFFX-BioC-5_at
## 5                                      AFFX-BioC-3_at
## 6                                     AFFX-BioDn-5_at
##   Composite.Element.Database.Entry.genbank.
## 1
## 2
## 3
## 4
## 5
## 6                                  NP_752788
##   Composite.Element.Database.Entry.ec. Composite.Element.Database.Entry.refseq.
## 1
## 2
## 3
## 4
## 5
## 6
##   Composite.Element.Database.Entry.swall.
## 1
## 2
## 3
## 4
## 5
## 6
##   Composite.Element.Database.Entry.ensembl.
## 1
## 2
## 3
## 4
## 5
## 6
##   Composite.Element.Database.Entry.go.
## 1
## 2
## 3
## 4
```

```
## 5
## 6
##   Composite.Element.Database.Entry.unigene.
## 1
## 2
## 3
## 4
## 5
## 6
##   Composite.Element.Database.Entry.mgd. Composite.Element.Database.Entry.locus.
## 1                                    NA                                      NA
## 2                                    NA                                      NA
## 3                                    NA                                      NA
## 4                                    NA                                      NA
## 5                                    NA                                      NA
## 6                                    NA                                      NA
##   Composite.Element.Database.Entry.pkr_hanks.
## 1
## 2
## 3
## 4
## 5
## 6
##   Composite.Element.Database.Entry.scop.
## 1
## 2
## 3
## 4
## 5
## 6
##   Composite.Element.Database.Entry.cp450.
## 1
## 2
## 3
## 4
## 5
## 6
```

```
annotation_MA2 <- annotation_MA2[sort(annotation_MA2$Composite.Element.Name,index.return=T)$ix,]

## Check if all probes are present in both sets
dim(annotation_MA2)
```

```
## [1] 45101    16
```

```
dim(LIMMAout2)
```

```
## [1] 45101     8
```

```
## Double check => "Assumption is the mother of all fuck up's ;)"
sum(annotation_MA2$Composite.Element.Name==sort(rownames(LIMMAout2)))
```

```
## [1] 45101
```

```
## Sort LIMMA output alphabetically on probe name
LIMMAout_sorted2 <- LIMMAout2[sort(rownames(LIMMAout2),index.return=T)$ix,]

## Add gene names to LIMMA output
LIMMAout_sorted2$gene <- annotation_MA2$Composite.Element.Database.Entry.ensembl.

LIMMAout_annot2 <- LIMMAout_sorted2[sort(LIMMAout_sorted2$adj.P.Val,index.return=T)$ix,]

#sort by adjusted p value from most significant to least
LIMMAout_sorted2 <- LIMMAout_sorted2[order(LIMMAout_sorted2$adj.P.Val, decreasing= F),]

#extract top 50 significant DE genes
LIMMAout_sorted2[1:50,]$gene
```

```
##  [1] "ENSMUSG00000025746" "ENSMUSG00000045303" "ENSMUSG00000045932"
##  [4] "ENSMUSG00000051925" "ENSMUSG00000034855" "ENSMUSG00000029419"
##  [7] "ENSMUSG00000023341" "ENSMUSG00000041827" "ENSMUSG00000022548"
## [10] ""                   "ENSMUSG00000047610" "ENSMUSG00000054261"
## [13] ""                   "ENSMUSG00000030107" "ENSMUSG00000025165"
## [16] "ENSMUSG00000020638" "ENSMUSG00000015947" "ENSMUSG00000022586"
## [19] "ENSMUSG00000027514" ""                   "ENSMUSG00000035152"
## [22] "ENSMUSG00000017830" "ENSMUSG00000029561" ""
## [25] "ENSMUSG00000048806" "ENSMUSG00000025498" "ENSMUSG00000035208"
## [28] "ENSMUSG00000022906" "ENSMUSG00000029379" ""
## [31] "ENSMUSG00000010358" "ENSMUSG00000039364" "ENSMUSG00000046031"
## [34] ""                   "ENSMUSG00000030921" "ENSMUSG00000019910"
## [37] ""                   "ENSMUSG00000001131" ""
## [40] "ENSMUSG00000003617" "ENSMUSG00000009670" "ENSMUSG00000028957"
## [43] "ENSMUSG00000023341" "ENSMUSG00000055116" "ENSMUSG00000015947"
## [46] ""                   ""                   ""
## [49] "ENSMUSG00000024371" ""
```

```
# Have a look at the results and search for other probesets for your DE genes
head(LIMMAout_annot2)
```

```
##               logFC   AveExpr        t      P.Value    adj.P.Val        B
## 1450297_at 5.716651  6.778338 44.60100 1.977975e-17 8.920867e-13 25.91879
## 1449025_at 3.251777 11.762997 37.08774 3.118352e-16 7.032040e-12 24.48160
## 1418293_at 3.318259 10.672902 29.69841 8.518266e-15 1.280608e-10 22.40893
## 1424518_at 4.104013  7.593314 26.49470 4.619705e-14 5.208833e-10 21.21036
## 1418930_at 6.303782  9.190682 24.97055 1.107804e-13 7.137579e-10 20.55617
## 1419697_at 3.816194  7.838359 25.43495 8.441607e-14 7.137579e-10 20.76185
##            diffexpressed diffexpressed_no_BH              gene
## 1450297_at            UP                  UP ENSMUSG00000025746
## 1449025_at            UP                  UP ENSMUSG00000045303
## 1418293_at            UP                  UP ENSMUSG00000045932
## 1424518_at            UP                  UP ENSMUSG00000051925
## 1418930_at            UP                  UP ENSMUSG00000034855
## 1419697_at            UP                  UP ENSMUSG00000029419
```

```
LIMMAout_annot2[LIMMAout_annot2$gene==" ENSMUSG00000025746",]
```

```
## [1] logFC               AveExpr             t
## [4] P.Value             adj.P.Val           B
## [7] diffexpressed       diffexpressed_no_BH gene
## <0 rows> (or 0-length row.names)
```

```
ensembl <- useEnsembl(biomart = "genes")
#listDatasets(ensembl)
searchDatasets(mart = ensembl, pattern = "musculus")
```

```
##                    dataset                    description     version
## 18  bmusculus_gene_ensembl Blue whale genes (mBalMus1.v2) mBalMus1.v2
## 107 mmusculus_gene_ensembl           Mouse genes (GRCm39)      GRCm39
```

```
#mmusculus_gene_ensembl
```

```
library('biomaRt')
mart <- useMart("ENSEMBL_MART_ENSEMBL")
mart <- useDataset("mmusculus_gene_ensembl", mart)

ensLookup <- gsub("\\.[0-9]*$", "", c(LIMMAout_sorted2$gene))

annotLookup <- getBM(
  mart=mart,
  attributes=c("ensembl_transcript_id", "ensembl_gene_id",
    "gene_biotype", "external_gene_name"),
  filter="ensembl_gene_id",
  values=ensLookup,
  uniqueRows=TRUE)

#retrieved external gene names
#unique(annotLookup$external_gene_name) #11449 genes

#retrieve the list of gene names from the limma output genes
gene_list <- unique(annotLookup$ensembl_gene_id[annotLookup$ensembl_gene_id %in% LIMMAout_sorted2$gene])
length(gene_list)
```

```
## [1] 11449
```

```
#extract DE genes
DEgeneIDs <- LIMMAout_sorted2$gene[LIMMAout_sorted2$adj.P.Val <= 0.05]
DEgeneIDs <- DEgeneIDs[DEgeneIDs != ""]
DEgeneIDs <- DEgeneIDs[DEgeneIDs%in%unique(annotLookup$ensembl_gene_id)]
length(DEgeneIDs)
```

```
## [1] 1382
```

```
#get gene symbols from ENSEMBL gene ids
DEgene_symbols2 <- NULL
for (gene in DEgeneIDs){
  n <- which(gene == gene_list)
  DEgene_symbols2 <- c(DEgene_symbols2, unique(annotLookup$external_gene_name)[n])
```

```
}

#top 10 DE genes
DEgene_symbol_and_ID <- cbind(DEgene_symbols2, DEgeneIDs)
head(DEgene_symbol_and_ID , 10)
```

```
##       DEgene_symbols2 DEgeneIDs
##  [1,] "Il6"           "ENSMUSG00000025746"
##  [2,] "Ifit2"         "ENSMUSG00000045932"
##  [3,] "Cxcl10"        "ENSMUSG00000034855"
##  [4,] "Ajm1"          "ENSMUSG00000029419"
##  [5,] "Mx2"           "ENSMUSG00000023341"
##  [6,] "Oasl1"         "ENSMUSG00000041827"
##  [7,] "Apod"          "ENSMUSG00000022548"
##  [8,] "Usp18"         "ENSMUSG00000030107"
##  [9,] "Sectm1a"       "ENSMUSG00000025165"
## [10,] "Cmpk2"         "ENSMUSG00000020638"
```

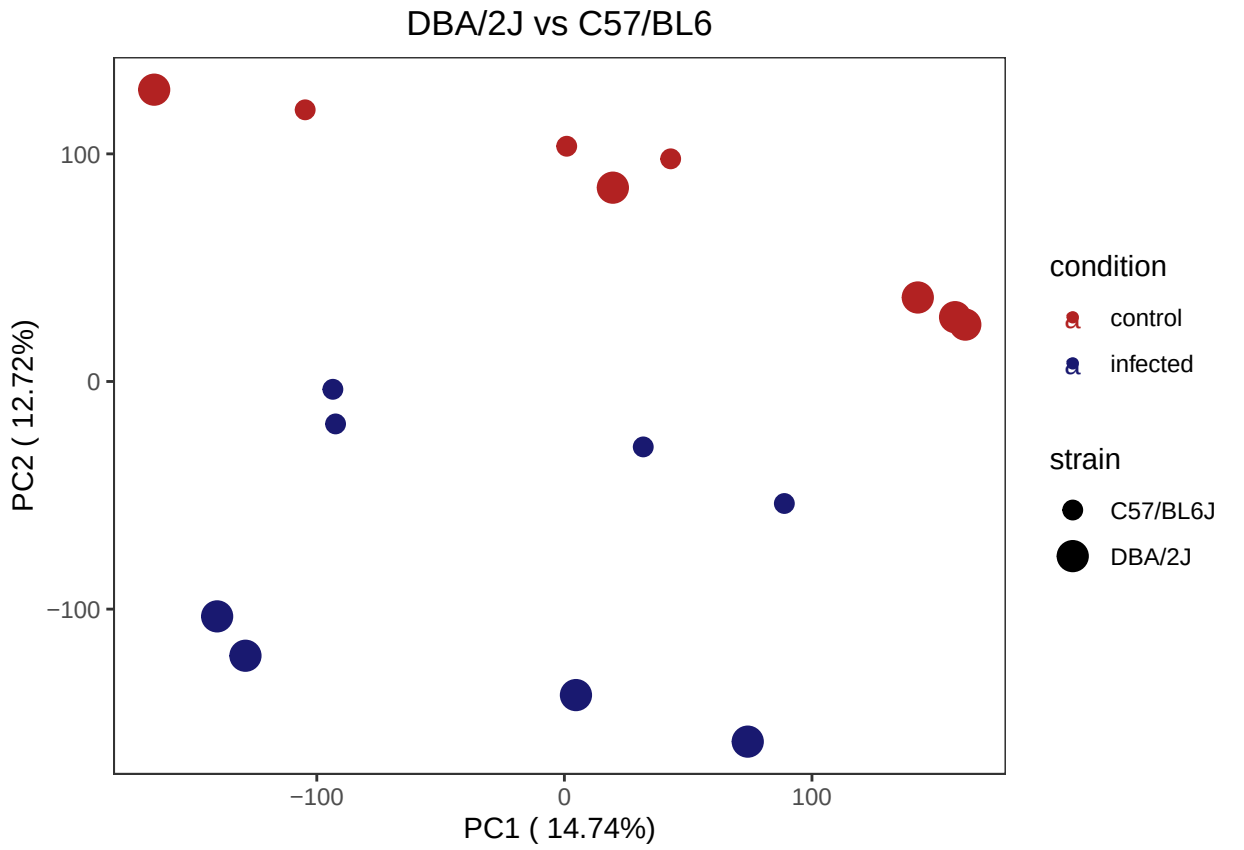
```
# transpose the data before PcA as this function requires the variables to b columns
data <- t(as.data.frame(miceRMA@assayData$exprs))
pca <- prcomp(data, center = T, scale. = T)

summary(pca)
```

```
## Importance of components:
##                             PC1     PC2     PC3      PC4     PC5      PC6
## Standard deviation     109.5792 94.5875 70.5787 58.29125 49.1200 45.97059
## Proportion of Variance   0.2662  0.1984  0.1105  0.07534  0.0535  0.04686
## Cumulative Proportion    0.2662  0.4646  0.5751  0.65040  0.7039  0.75075
##                             PC7      PC8      PC9     PC10     PC11     PC12
## Standard deviation     42.54130 41.32187 37.95470 35.78304 34.98692 32.96254
## Proportion of Variance  0.04013  0.03786  0.03194  0.02839  0.02714  0.02409
## Cumulative Proportion   0.79088  0.82874  0.86068  0.88907  0.91621  0.94030
##                            PC13     PC14     PC15      PC16
## Standard deviation     31.05439 30.60996 28.12686 4.935e-13
## Proportion of Variance  0.02138  0.02077  0.01754 0.000e+00
## Cumulative Proportion   0.96168  0.98246  1.00000 1.000e+00
```

```
# save as dataframe and add treatment variable
pca_out <- as.data.frame(pca$x)

pca_out$condition <- as.character(miceRMA@phenoData@data$condition)

# get lablels
percentage <- round(pca$sdev / sum(pca$sdev) * 100, 2)
percentage <- paste( colnames(pca_out), "(", paste( as.character(percentage), "%", ")", sep="") )

ggplot(data = pca_out)+
  ggtitle("DBA/2J vs C57/BL6")+
  geom_point(aes(x = PC1, y = PC2, colour = condition, label='', size=strain))+
  geom_text(aes(x = PC1, y = PC2, colour = condition, label=''),hjust=0.5, vjust=1.15)+
  theme_bw()+
```

```
  xlab(percentage[1])+
  ylab(percentage[2])+
  labs(colour = "condition")+
  theme(plot.title = element_text(hjust = 0.5))+
  scale_size_manual(values = c(3, 5))+
  scale_colour_manual(values = c("firebrick", "midnightblue"))+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
```

```
## Warning: Ignoring unknown aesthetics: label
```




```
#ggsave("PCA_array2_bothstrains.png", dpi=750, width=8, height = 5)
```

RNAseq dataset E-MTAB-5337

RNA-seq of whole lungs from Irgm1-/- and wildtype littermates mice that were either uninfected or infected with influenza for 10 days. Here, we only used wild type 3 infected vs 3 non-infected samples. For each sample, two technical replicates were present (total 12)

```
setwd("/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Datase
```

```
## Get file locations
#ERR1753616 - ERR1753621 : WT, non-infected
#ERR1753622- ERR1753627: WT, infected 10 dpi
```

```r
files1 <- c("/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/
            "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da
           "/Users/seoyeon/Desktop/MSc Bioinformatics Year 1/Applied High-throughput Analysis/Project/Da

tx2gene1 <- read.delim("./mus_musculus_trans2gen.txt")
#tx2gene1

names(files1) <- paste0("sample", 1:12)
txi.kallisto <- tximport(files1, type = "kallisto", txOut = FALSE, tx2gene=tx2gene1)
```

```
## Note: importing ‘abundance.h5‘ is typically faster than ‘abundance.tsv‘

## reading in files with read_tsv

## 1 2 3 4 5 6 7 8 9 10 11 12
## transcripts missing from tx2gene: 1673
## summarizing abundance
## summarizing counts
## summarizing length
```

```r
dim(txi.kallisto$counts)
```

```
## [1] 36047    12
```

```r
head(txi.kallisto$length)
```

```
##                          sample1  sample2   sample3   sample4  sample5   sample6
## ENSMUSG00000000001.4   3213.0000 3213.000 3213.0000 3213.0000 3213.000 3213.0000
## ENSMUSG00000000003.15   750.5000  750.500  750.5000  750.5000  750.500  750.5000
## ENSMUSG00000000028.15 1495.8714 1480.561 1462.6724 1460.3967 1465.040 1479.9446
## ENSMUSG00000000037.16 1638.0000 2340.002 2573.9997 3276.0010 3602.508 3391.0000
## ENSMUSG00000000049.11  443.4987  433.752  443.4987  443.4987  318.000  497.3792
## ENSMUSG00000000056.7  3443.1809 3146.751 2284.8596 2217.8745 2907.992 2752.3972
##                          sample7   sample8  sample9  sample10 sample11 sample12
## ENSMUSG00000000001.4   3213.0000 3213.0000 3213.000 3213.0000 3213.000 3213.000
## ENSMUSG00000000003.15   750.5000  750.5000  750.500  750.5000  750.500  750.500
## ENSMUSG00000000028.15 1540.7317 1677.0540 1743.725 1699.3468 1683.682 1591.334
## ENSMUSG00000000037.16 2808.0003 2807.9994 3651.758 2807.9998 3442.499 3510.001
## ENSMUSG00000000049.11  443.4987  497.3788  318.000  635.9999  682.000  318.000
## ENSMUSG00000000056.7  2890.5020 4067.4717 3477.942 3140.7988 3472.230 2859.385
```

118489 features 12 samples in trascript level.36047 features in gene level.

```
sdrf <- read.delim("./RNAseq/E-MTAB-5337.sdrf.txt")
head(sdrf)
```

```
##                 Source.Name Comment.ENA_SAMPLE. Comment.BioSD_SAMPLE.
## 1 Infected_Irgm1-/-_Day10_1          ERS1471225         SAMEA24832918
## 2 Infected_Irgm1-/-_Day10_1          ERS1471225         SAMEA24832918
## 3 Infected_Irgm1-/-_Day10_2          ERS1471226         SAMEA24833668
## 4 Infected_Irgm1-/-_Day10_2          ERS1471226         SAMEA24833668
## 5 Infected_Irgm1-/-_Day10_3          ERS1471227         SAMEA24834418
## 6 Infected_Irgm1-/-_Day10_3          ERS1471227         SAMEA24834418
##   Characteristics.organism. Characteristics.strain. Characteristics.age.
## 1              Mus musculus                C57BL/6              8 to 12
## 2              Mus musculus                C57BL/6              8 to 12
## 3              Mus musculus                C57BL/6              8 to 12
## 4              Mus musculus                C57BL/6              8 to 12
## 5              Mus musculus                C57BL/6              8 to 12
## 6              Mus musculus                C57BL/6              8 to 12
##   Unit.time.unit. Characteristics.genotype.
## 1            week         Irgm1-/- knockout
## 2            week         Irgm1-/- knockout
## 3            week         Irgm1-/- knockout
## 4            week         Irgm1-/- knockout
## 5            week         Irgm1-/- knockout
## 6            week         Irgm1-/- knockout
##                   Characteristics.phenotype. Characteristics.organism.part.
## 1 protected from influenza-induced mortality                           lung
## 2 protected from influenza-induced mortality                           lung
## 3 protected from influenza-induced mortality                           lung
## 4 protected from influenza-induced mortality                           lung
## 5 protected from influenza-induced mortality                           lung
## 6 protected from influenza-induced mortality                           lung
##   Material.Type Protocol.REF    Performer Protocol.REF.1  Performer.1
## 1 organism part P-MTAB-53254 Ashley Steed   P-MTAB-53248 Ashley Steed
## 2 organism part P-MTAB-53254 Ashley Steed   P-MTAB-53248 Ashley Steed
## 3 organism part P-MTAB-53254 Ashley Steed   P-MTAB-53248 Ashley Steed
## 4 organism part P-MTAB-53254 Ashley Steed   P-MTAB-53248 Ashley Steed
## 5 organism part P-MTAB-53254 Ashley Steed   P-MTAB-53248 Ashley Steed
## 6 organism part P-MTAB-53254 Ashley Steed   P-MTAB-53248 Ashley Steed
##   Protocol.REF.2  Performer.2 Protocol.REF.3
## 1   P-MTAB-53249 Ashley Steed   P-MTAB-53250
## 2   P-MTAB-53249 Ashley Steed   P-MTAB-53250
## 3   P-MTAB-53249 Ashley Steed   P-MTAB-53250
## 4   P-MTAB-53249 Ashley Steed   P-MTAB-53250
## 5   P-MTAB-53249 Ashley Steed   P-MTAB-53250
## 6   P-MTAB-53249 Ashley Steed   P-MTAB-53250
##                                           Performer.3
## 1 Genome Technology Access Center at Washington University
## 2 Genome Technology Access Center at Washington University
## 3 Genome Technology Access Center at Washington University
## 4 Genome Technology Access Center at Washington University
## 5 Genome Technology Access Center at Washington University
## 6 Genome Technology Access Center at Washington University
##                Extract.Name Comment.LIBRARY_LAYOUT. Comment.LIBRARY_SELECTION.
```

```
## 1 Infected_Irgm1-/-_Day10_1                  SINGLE               Inverse rRNA
## 2 Infected_Irgm1-/-_Day10_1                  SINGLE               Inverse rRNA
## 3 Infected_Irgm1-/-_Day10_2                  SINGLE               Inverse rRNA
## 4 Infected_Irgm1-/-_Day10_2                  SINGLE               Inverse rRNA
## 5 Infected_Irgm1-/-_Day10_3                  SINGLE               Inverse rRNA
## 6 Infected_Irgm1-/-_Day10_3                  SINGLE               Inverse rRNA
##   Comment.LIBRARY_SOURCE. Comment.LIBRARY_STRAND. Comment.LIBRARY_STRATEGY.
## 1          TRANSCRIPTOMIC          not applicable                   RNA-Seq
## 2          TRANSCRIPTOMIC          not applicable                   RNA-Seq
## 3          TRANSCRIPTOMIC          not applicable                   RNA-Seq
## 4          TRANSCRIPTOMIC          not applicable                   RNA-Seq
## 5          TRANSCRIPTOMIC          not applicable                   RNA-Seq
## 6          TRANSCRIPTOMIC          not applicable                   RNA-Seq
##   Protocol.REF.4                                          Performer.4
## 1   P-MTAB-53251 Genome Technology Access Center at Washington University
## 2   P-MTAB-53251 Genome Technology Access Center at Washington University
## 3   P-MTAB-53251 Genome Technology Access Center at Washington University
## 4   P-MTAB-53251 Genome Technology Access Center at Washington University
## 5   P-MTAB-53251 Genome Technology Access Center at Washington University
## 6   P-MTAB-53251 Genome Technology Access Center at Washington University
##                   Assay.Name Comment.technical.replicate.group.
## 1   Infected_Irgm1-/-_Day10_1                            group 24
## 2 Infected_Irgm1-/-_Day10_1_1                            group 24
## 3   Infected_Irgm1-/-_Day10_2                            group 23
## 4 Infected_Irgm1-/-_Day10_2_1                            group 23
## 5   Infected_Irgm1-/-_Day10_3                            group 22
## 6 Infected_Irgm1-/-_Day10_3_1                            group 22
##    Technology.Type Comment.ENA_EXPERIMENT.
## 1 sequencing assay              ERX1820995
## 2 sequencing assay              ERX1820995
## 3 sequencing assay              ERX1820996
## 4 sequencing assay              ERX1820996
## 5 sequencing assay              ERX1820997
## 6 sequencing assay              ERX1820997
##                                        Scan.Name
## 1 run_1653_s_3_withindex_sequence.txt_CGAAAGT.fq.gz
## 2 run_1653_s_4_withindex_sequence.txt_CGAAAGT.fq.gz
## 3 run_1653_s_3_withindex_sequence.txt_CAGAGTC.fq.gz
## 4 run_1653_s_4_withindex_sequence.txt_CAGAGTC.fq.gz
## 5 run_1653_s_3_withindex_sequence.txt_ACTGGAT.fq.gz
## 6 run_1653_s_4_withindex_sequence.txt_ACTGGAT.fq.gz
##                       Comment.SUBMITTED_FILE_NAME. Comment.ENA_RUN.
## 1 run_1653_s_3_withindex_sequence.txt_CGAAAGT.fq.gz       ERR1753604
## 2 run_1653_s_4_withindex_sequence.txt_CGAAAGT.fq.gz       ERR1753605
## 3 run_1653_s_3_withindex_sequence.txt_CAGAGTC.fq.gz       ERR1753606
## 4 run_1653_s_4_withindex_sequence.txt_CAGAGTC.fq.gz       ERR1753607
## 5 run_1653_s_3_withindex_sequence.txt_ACTGGAT.fq.gz       ERR1753608
## 6 run_1653_s_4_withindex_sequence.txt_ACTGGAT.fq.gz       ERR1753609
##                                                         Comment.FASTQ_URI.
## 1 ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR175/004/ERR1753604/ERR1753604.fastq.gz
## 2 ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR175/005/ERR1753605/ERR1753605.fastq.gz
## 3 ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR175/006/ERR1753606/ERR1753606.fastq.gz
## 4 ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR175/007/ERR1753607/ERR1753607.fastq.gz
## 5 ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR175/008/ERR1753608/ERR1753608.fastq.gz
```

```
## 6 ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR175/009/ERR1753609/ERR1753609.fastq.gz
##   Protocol.REF.5                                                Performer.5
## 1   P-MTAB-53252 Genome Technology Access Center at Washington University
## 2   P-MTAB-53252 Genome Technology Access Center at Washington University
## 3   P-MTAB-53252 Genome Technology Access Center at Washington University
## 4   P-MTAB-53252 Genome Technology Access Center at Washington University
## 5   P-MTAB-53252 Genome Technology Access Center at Washington University
## 6   P-MTAB-53252 Genome Technology Access Center at Washington University
##   Protocol.REF.6                                                Performer.6
## 1   P-MTAB-53253 Genome Technology Access Center at Washington University
## 2   P-MTAB-53253 Genome Technology Access Center at Washington University
## 3   P-MTAB-53253 Genome Technology Access Center at Washington University
## 4   P-MTAB-53253 Genome Technology Access Center at Washington University
## 5   P-MTAB-53253 Genome Technology Access Center at Washington University
## 6   P-MTAB-53253 Genome Technology Access Center at Washington University
##                  Derived.Array.Data.File
## 1 all.gene_counts_D0-and-D10-samples.txt
## 2 all.gene_counts_D0-and-D10-samples.txt
## 3 all.gene_counts_D0-and-D10-samples.txt
## 4 all.gene_counts_D0-and-D10-samples.txt
## 5 all.gene_counts_D0-and-D10-samples.txt
## 6 all.gene_counts_D0-and-D10-samples.txt
##                                                          Comment..Derived.ArrayExpress.FTI
## 1 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 2 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 3 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 4 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 5 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 6 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
##   Protocol.REF.7                                                Performer.7
## 1   P-MTAB-53252 Genome Technology Access Center at Washington University
## 2   P-MTAB-53252 Genome Technology Access Center at Washington University
## 3   P-MTAB-53252 Genome Technology Access Center at Washington University
## 4   P-MTAB-53252 Genome Technology Access Center at Washington University
## 5   P-MTAB-53252 Genome Technology Access Center at Washington University
## 6   P-MTAB-53252 Genome Technology Access Center at Washington University
##   Protocol.REF.8                                                Performer.8
## 1   P-MTAB-53253 Genome Technology Access Center at Washington University
## 2   P-MTAB-53253 Genome Technology Access Center at Washington University
## 3   P-MTAB-53253 Genome Technology Access Center at Washington University
## 4   P-MTAB-53253 Genome Technology Access Center at Washington University
## 5   P-MTAB-53253 Genome Technology Access Center at Washington University
## 6   P-MTAB-53253 Genome Technology Access Center at Washington University
##                      Derived.Array.Data.File.1
## 1 all.transcript_counts_D0-and-D10-samples.txt
## 2 all.transcript_counts_D0-and-D10-samples.txt
## 3 all.transcript_counts_D0-and-D10-samples.txt
## 4 all.transcript_counts_D0-and-D10-samples.txt
## 5 all.transcript_counts_D0-and-D10-samples.txt
## 6 all.transcript_counts_D0-and-D10-samples.txt
##                                                        Comment..Derived.ArrayExpress.FTP.f
## 1 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 2 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 3 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
```

```
## 4 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 5 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 6 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
##   Protocol.REF.9                                                Performer.9
## 1   P-MTAB-53252 Genome Technology Access Center at Washington University
## 2   P-MTAB-53252 Genome Technology Access Center at Washington University
## 3   P-MTAB-53252 Genome Technology Access Center at Washington University
## 4   P-MTAB-53252 Genome Technology Access Center at Washington University
## 5   P-MTAB-53252 Genome Technology Access Center at Washington University
## 6   P-MTAB-53252 Genome Technology Access Center at Washington University
##   Protocol.REF.10                                               Performer.10
## 1    P-MTAB-53253 Genome Technology Access Center at Washington University
## 2    P-MTAB-53253 Genome Technology Access Center at Washington University
## 3    P-MTAB-53253 Genome Technology Access Center at Washington University
## 4    P-MTAB-53253 Genome Technology Access Center at Washington University
## 5    P-MTAB-53253 Genome Technology Access Center at Washington University
## 6    P-MTAB-53253 Genome Technology Access Center at Washington University
##                          Derived.Array.Data.File.2
## 1 all.gene_moderated_log2cpm_D0-and-D10-samples.txt
## 2 all.gene_moderated_log2cpm_D0-and-D10-samples.txt
## 3 all.gene_moderated_log2cpm_D0-and-D10-samples.txt
## 4 all.gene_moderated_log2cpm_D0-and-D10-samples.txt
## 5 all.gene_moderated_log2cpm_D0-and-D10-samples.txt
## 6 all.gene_moderated_log2cpm_D0-and-D10-samples.txt
##                                                                 Comment..Derived.ArrayExpress.FTP.
## 1 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 2 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 3 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 4 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 5 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
## 6 ftp://ftp.ebi.ac.uk/pub/databases/microarray/data/experiment/MTAB/E-MTAB-5337/E-MTAB-5337.processed
##   Factor.Value.genotype. Factor.Value.infect. Factor.Value.sampling.time.point.
## 1      Irgm1-/- knockout   influenza A (H1N1)                                 10
## 2      Irgm1-/- knockout   influenza A (H1N1)                                 10
## 3      Irgm1-/- knockout   influenza A (H1N1)                                 10
## 4      Irgm1-/- knockout   influenza A (H1N1)                                 10
## 5      Irgm1-/- knockout   influenza A (H1N1)                                 10
## 6      Irgm1-/- knockout   influenza A (H1N1)                                 10
##   Unit.time.unit..1
## 1               day
## 2               day
## 3               day
## 4               day
## 5               day
## 6               day
```

```
print(sdrf[,c("Source.Name","Technology.Type", "Characteristics.phenotype.", "Characteristics.organism.p
```

```
##                   Source.Name  Technology.Type
## 1   Infected_Irgm1-/-_Day10_1 sequencing assay
## 2   Infected_Irgm1-/-_Day10_1 sequencing assay
## 3   Infected_Irgm1-/-_Day10_2 sequencing assay
## 4   Infected_Irgm1-/-_Day10_2 sequencing assay
## 5   Infected_Irgm1-/-_Day10_3 sequencing assay
```

```
## 6   Infected_Irgm1-/-_Day10_3 sequencing assay
## 7  Uninfected_Irgm1-/-_Day0_1 sequencing assay
## 8  Uninfected_Irgm1-/-_Day0_1 sequencing assay
## 9  Uninfected_Irgm1-/-_Day0_2 sequencing assay
## 10 Uninfected_Irgm1-/-_Day0_2 sequencing assay
## 11 Uninfected_Irgm1-/-_Day0_3 sequencing assay
## 12 Uninfected_Irgm1-/-_Day0_3 sequencing assay
## 13       Uninfected_WT_Day0_1 sequencing assay
## 14       Uninfected_WT_Day0_1 sequencing assay
## 15       Uninfected_WT_Day0_2 sequencing assay
## 16       Uninfected_WT_Day0_2 sequencing assay
## 17       Uninfected_WT_Day0_3 sequencing assay
## 18       Uninfected_WT_Day0_3 sequencing assay
## 19        Infected_WT_Day10_1 sequencing assay
## 20        Infected_WT_Day10_1 sequencing assay
## 21        Infected_WT_Day10_2 sequencing assay
## 22        Infected_WT_Day10_2 sequencing assay
## 23        Infected_WT_Day10_3 sequencing assay
## 24        Infected_WT_Day10_3 sequencing assay
## 25   Infected_Irgm1-/-_Day3_1 sequencing assay
## 26   Infected_Irgm1-/-_Day3_1 sequencing assay
## 27   Infected_Irgm1-/-_Day3_2 sequencing assay
## 28   Infected_Irgm1-/-_Day3_2 sequencing assay
## 29   Infected_Irgm1-/-_Day3_3 sequencing assay
## 30   Infected_Irgm1-/-_Day3_3 sequencing assay
## 31   Infected_Irgm1-/-_Day6_1 sequencing assay
## 32   Infected_Irgm1-/-_Day6_1 sequencing assay
## 33   Infected_Irgm1-/-_Day6_2 sequencing assay
## 34   Infected_Irgm1-/-_Day6_2 sequencing assay
## 35   Infected_Irgm1-/-_Day6_3 sequencing assay
## 36   Infected_Irgm1-/-_Day6_3 sequencing assay
## 37         Infected_WT_Day3_1 sequencing assay
## 38         Infected_WT_Day3_1 sequencing assay
## 39         Infected_WT_Day3_2 sequencing assay
## 40         Infected_WT_Day3_2 sequencing assay
## 41         Infected_WT_Day3_3 sequencing assay
## 42         Infected_WT_Day3_3 sequencing assay
## 43         Infected_WT_Day6_1 sequencing assay
## 44         Infected_WT_Day6_1 sequencing assay
## 45         Infected_WT_Day6_2 sequencing assay
## 46         Infected_WT_Day6_2 sequencing assay
## 47         Infected_WT_Day6_3 sequencing assay
## 48         Infected_WT_Day6_3 sequencing assay
##                    Characteristics.phenotype. Characteristics.organism.part.
## 1  protected from influenza-induced mortality                            lung
## 2  protected from influenza-induced mortality                            lung
## 3  protected from influenza-induced mortality                            lung
## 4  protected from influenza-induced mortality                            lung
## 5  protected from influenza-induced mortality                            lung
## 6  protected from influenza-induced mortality                            lung
## 7  protected from influenza-induced mortality                            lung
## 8  protected from influenza-induced mortality                            lung
## 9  protected from influenza-induced mortality                            lung
## 10 protected from influenza-induced mortality                            lung
```

```
## 11 protected from influenza-induced mortality                          lung
## 12 protected from influenza-induced mortality                          lung
## 13 susceptible to influenza-induced mortality                          lung
## 14 susceptible to influenza-induced mortality                          lung
## 15 susceptible to influenza-induced mortality                          lung
## 16 susceptible to influenza-induced mortality                          lung
## 17 susceptible to influenza-induced mortality                          lung
## 18 susceptible to influenza-induced mortality                          lung
## 19 susceptible to influenza-induced mortality                          lung
## 20 susceptible to influenza-induced mortality                          lung
## 21 susceptible to influenza-induced mortality                          lung
## 22 susceptible to influenza-induced mortality                          lung
## 23 susceptible to influenza-induced mortality                          lung
## 24 susceptible to influenza-induced mortality                          lung
## 25 protected from influenza-induced mortality                          lung
## 26 protected from influenza-induced mortality                          lung
## 27 protected from influenza-induced mortality                          lung
## 28 protected from influenza-induced mortality                          lung
## 29 protected from influenza-induced mortality                          lung
## 30 protected from influenza-induced mortality                          lung
## 31 protected from influenza-induced mortality                          lung
## 32 protected from influenza-induced mortality                          lung
## 33 protected from influenza-induced mortality                          lung
## 34 protected from influenza-induced mortality                          lung
## 35 protected from influenza-induced mortality                          lung
## 36 protected from influenza-induced mortality                          lung
## 37 susceptible to influenza-induced mortality                          lung
## 38 susceptible to influenza-induced mortality                          lung
## 39 susceptible to influenza-induced mortality                          lung
## 40 susceptible to influenza-induced mortality                          lung
## 41 susceptible to influenza-induced mortality                          lung
## 42 susceptible to influenza-induced mortality                          lung
## 43 susceptible to influenza-induced mortality                          lung
## 44 susceptible to influenza-induced mortality                          lung
## 45 susceptible to influenza-induced mortality                          lung
## 46 susceptible to influenza-induced mortality                          lung
## 47 susceptible to influenza-induced mortality                          lung
## 48 susceptible to influenza-induced mortality                          lung
```

```
#get annotation data
mart = useMart(host="useast.ensembl.org",
                biomart="ENSEMBL_MART_ENSEMBL",
                dataset="mmusculus_gene_ensembl")

mmusculus <- getBM(attributes=c('ensembl_transcript_id',
                                'ensembl_gene_id',
                                'external_gene_name'),
                        mart = mart)
head(mmusculus)
```

```
##   ensembl_transcript_id    ensembl_gene_id external_gene_name
## 1    ENSMUST00000082387 ENSMUSG00000064336              mt-Tf
## 2    ENSMUST00000082388 ENSMUSG00000064337            mt-Rnr1
## 3    ENSMUST00000082389 ENSMUSG00000064338              mt-Tv
```

```
## 4    ENSMUST00000082390 ENSMUSG00000064339            mt-Rnr2
## 5    ENSMUST00000082391 ENSMUSG00000064340             mt-Tl1
## 6    ENSMUST00000082392 ENSMUSG00000064341             mt-Nd1
```

```
# What are the available attributes
atr <- listAttributes(mart)

data <- getBM(attributes = c('ensembl_gene_id', 'ensembl_transcript_id',
                             'external_gene_name'),
              mart = mart)
```

###Which samples we are using, and not using: We will only be using samples involving susceptible (Wild Type) after 10 days of infection with influenza.

Check for duplicate rows

```
# no duplicate rows
sum(duplicated(rownames(txi.kallisto$counts)))
```

```
## [1] 0
```

```
setwd("./RNAseq_output")
```

```
## Make tpm values compatible with edgeR
cts <- txi.kallisto$counts
normMat <- txi.kallisto$length
head(normMat)
```

```
##                         sample1  sample2   sample3   sample4  sample5   sample6
## ENSMUSG00000000001.4  3213.0000 3213.000 3213.0000 3213.0000 3213.000 3213.0000
## ENSMUSG00000000003.15  750.5000  750.500  750.5000  750.5000  750.500  750.5000
## ENSMUSG00000000028.15 1495.8714 1480.561 1462.6724 1460.3967 1465.040 1479.9446
## ENSMUSG00000000037.16 1638.0000 2340.002 2573.9997 3276.0010 3602.508 3391.0000
## ENSMUSG00000000049.11  443.4987  433.752  443.4987  443.4987  318.000  497.3792
## ENSMUSG00000000056.7  3443.1809 3146.751 2284.8596 2217.8745 2907.992 2752.3972
##                         sample7   sample8  sample9  sample10 sample11 sample12
## ENSMUSG00000000001.4  3213.0000 3213.0000 3213.000 3213.0000 3213.000 3213.000
## ENSMUSG00000000003.15  750.5000  750.5000  750.500  750.5000  750.500  750.500
## ENSMUSG00000000028.15 1540.7317 1677.0540 1743.725 1699.3468 1683.682 1591.334
## ENSMUSG00000000037.16 2808.0003 2807.9994 3651.758 2807.9998 3442.499 3510.001
## ENSMUSG00000000049.11  443.4987  497.3788  318.000  635.9999  682.000  318.000
## ENSMUSG00000000056.7  2890.5020 4067.4717 3477.942 3140.7988 3472.230 2859.385
```

```
# Obtaining per-observation scaling factors for length, adjusted to avoid
# changing the magnitude of the counts.
normMat <- normMat/exp(rowMeans(log(normMat)))
normCts <- cts/normMat
head(normCts)
```

```
##                         sample1    sample2  sample3    sample4    sample5
## ENSMUSG00000000001.4  686.0000 618.000000 730.0000 679.000000 618.000000
## ENSMUSG00000000003.15   0.0000   0.000000   0.0000   0.000000   0.000000
## ENSMUSG00000000028.15 153.4060 121.642167 231.9512 204.354916 178.065788
## ENSMUSG00000000037.16  12.4827   6.241345  12.4827   6.241352   4.054054
## ENSMUSG00000000049.11   0.0000   2.044942   0.0000   0.000000   2.789300
## ENSMUSG00000000056.7  158.4009 176.174112 222.8767 225.495887 150.201141
##                         sample6    sample7    sample8    sample9  sample10
## ENSMUSG00000000001.4  586.000000 603.000000 610.000000 1038.00000 991.00000
## ENSMUSG00000000003.15   0.000000   0.000000   0.000000    0.00000   0.00000
## ENSMUSG00000000028.15 223.130622 106.616330  55.786146  170.16046 181.10422
## ENSMUSG00000000037.16   4.306915   6.241344  12.482701   11.19825  12.48269
## ENSMUSG00000000049.11   1.783343   0.000000   1.783344    1.39465   1.39465
## ENSMUSG00000000056.7  133.225844 202.129337 125.939701  188.81850 203.66169
##                         sample11  sample12
## ENSMUSG00000000001.4  584.0000000 621.00000
## ENSMUSG00000000003.15   0.0000000   0.00000
## ENSMUSG00000000028.15 147.9495762 125.32669
## ENSMUSG00000000037.16   6.7879752  12.48269
## ENSMUSG00000000049.11   0.6502914   1.39465
## ENSMUSG00000000056.7  144.0585049 162.63249
```

```
# Computing effective library sizes from scaled counts, to account for
# composition biases between samples.
eff.lib <- calcNormFactors(normCts) * colSums(normCts)
head(eff.lib)
```

```
## sample1 sample2 sample3 sample4 sample5 sample6
## 6809954 6625648 7294221 7122479 6301408 6145094
```

```
# Combining effective library sizes with the length factors, and calculating
# offsets for a log-link GLM.

#merge every two columns which correspond to each 2 technical replicates
merged_normMat <- NULL
for (x in seq(1,12,2)){
  merged_normMat <- cbind(merged_normMat,normMat[,x]+normMat[,x+1])
}

normMat <- merged_normMat
normMat <- sweep(normMat, 2, eff.lib, "*")
```

```
## Warning in sweep(normMat, 2, eff.lib, "*"): STATS is longer than the extent of
## 'dim(x)[MARGIN]'
```

```
normMat <- log(normMat)

new_cts <- NULL
for (c in seq(1,12,2)){
  new_cts <- cbind(new_cts, cts[,c]+cts[,c+1])
}
cts <- new_cts
```

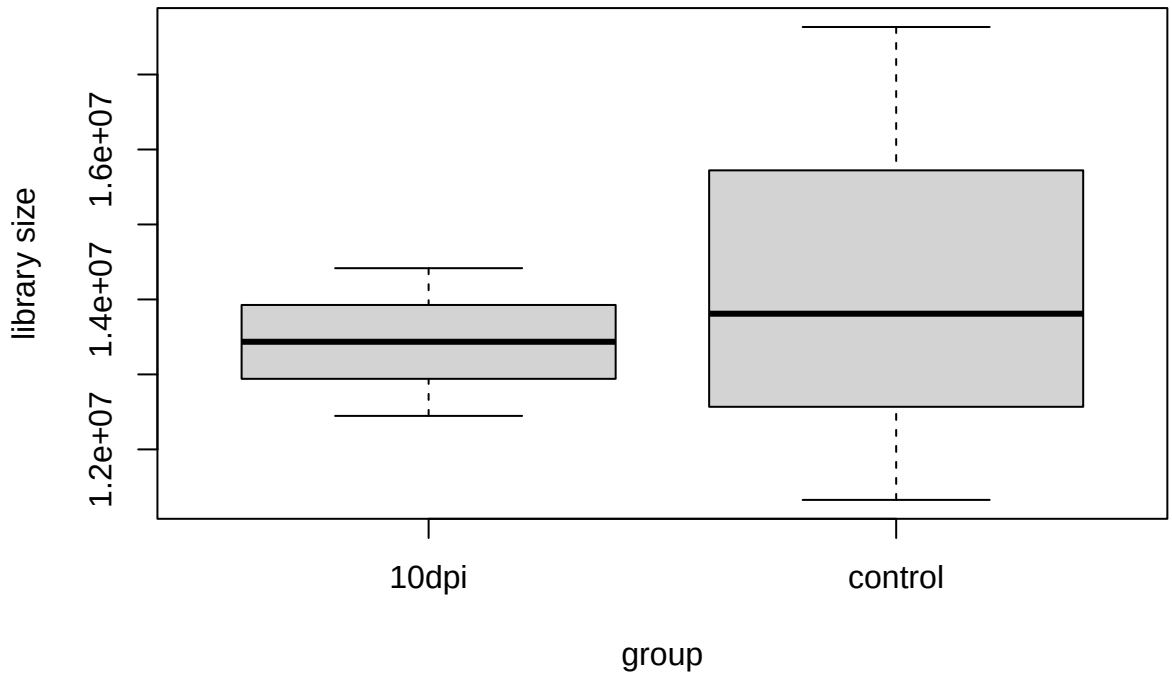
```
y <- DGEList(cts)
y <- scaleOffset(y, normMat)
head(y)
```

```
## An object of class "DGEList"
## $counts
##                          Sample1    Sample2   Sample3   Sample4     Sample5
## ENSMUSG00000000001.4  1304.00000 1409.00000 1204.0000 1213.0000 2029.000000
## ENSMUSG00000000003.15    0.00000    0.00000    0.0000    0.0000    0.000000
## ENSMUSG00000000028.15  262.26604  408.34856  378.5002  165.0942  387.066300
## ENSMUSG00000000037.16   12.00001   18.00001   10.0000   18.0000   26.000006
## ENSMUSG00000000049.11    2.00000    0.00000    4.0000    2.0000    2.999999
## ENSMUSG00000000056.7   364.97800  334.97230  266.6449  363.8936  430.216440
##                          Sample6
## ENSMUSG00000000001.4  1205.0000
## ENSMUSG00000000003.15    0.0000
## ENSMUSG00000000028.15  287.2151
## ENSMUSG00000000037.16   23.0000
## ENSMUSG00000000049.11    2.0000
## ENSMUSG00000000056.7   320.3270
##
## $samples
##         group lib.size norm.factors
## Sample1     1 13413997            1
## Sample2     1 14385696            1
## Sample3     1 12362925            1
## Sample4     1 13767983            1
## Sample5     1 18090430            1
## Sample6     1 11097351            1
##
## $offset
##                           [,1]     [,2]     [,3]     [,4]     [,5]     [,6]
## ENSMUSG00000000001.4  16.44816 16.42072 16.51685 16.49303 16.37055 16.34543
## ENSMUSG00000000003.15 16.42970 16.40437 16.66884 16.65421 16.22957 16.20804
## ENSMUSG00000000028.15 16.39974 16.35421 16.45782 16.52258 16.46777 16.39261
## ENSMUSG00000000037.16 16.04006 16.40039 16.84341 16.60941 16.32474 16.37672
## ENSMUSG00000000049.11 16.41103 16.39464 16.40659 16.52592 16.41729 16.43926
## ENSMUSG00000000056.7  16.51539 16.10921 16.60249 16.79426 16.31963 16.25375
```

```
## Library sizes
infection <- factor(c("10dpi","10dpi","10dpi","control", "control", "control"))
lib <- NULL
n <- 1
for (x in seq(1,12,2)){
  lib[n] <- eff.lib[x]+ eff.lib[x+1]
  n <- n + 1
}
lib
```

```
## [1] 13435602 14416700 12446502 13809810 17633891 11326350
```

```
#jpeg("library_sizes_musmusculus.jpg")
boxplot(lib~as.factor(infection),xlab="group",ylab="library size")
```



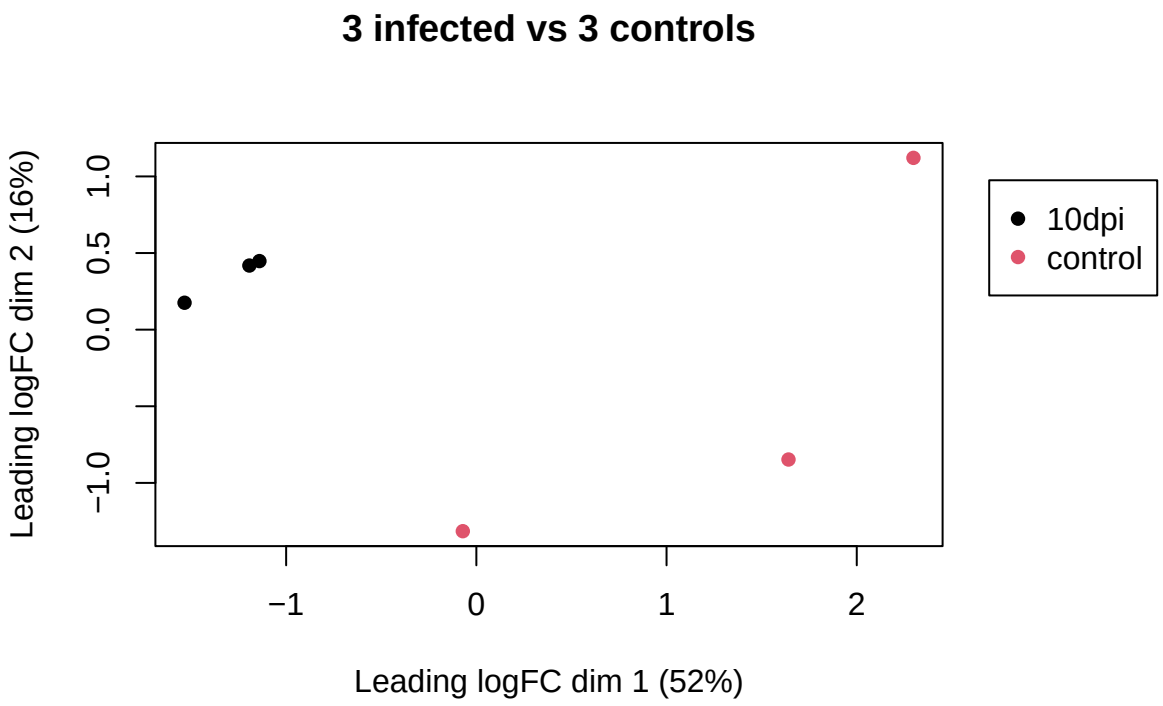

```
#dev.off()

wilcox.test(lib~as.factor(infection))
```

```
##
##  Wilcoxon rank sum exact test
##
## data:  lib by as.factor(infection)
## W = 4, p-value = 1
## alternative hypothesis: true location shift is not equal to 0
```

```
## Optionally filter on counts mean
cutoff <- 3/(mean(y$samples$lib.size)/1000000)
keep <- rowSums(cpm(y)>cutoff) >= 3
y <- y[keep,  ,keep.lib.sizes=FALSE]
summary(keep) #FALSE 15672 TRUE 20375
```

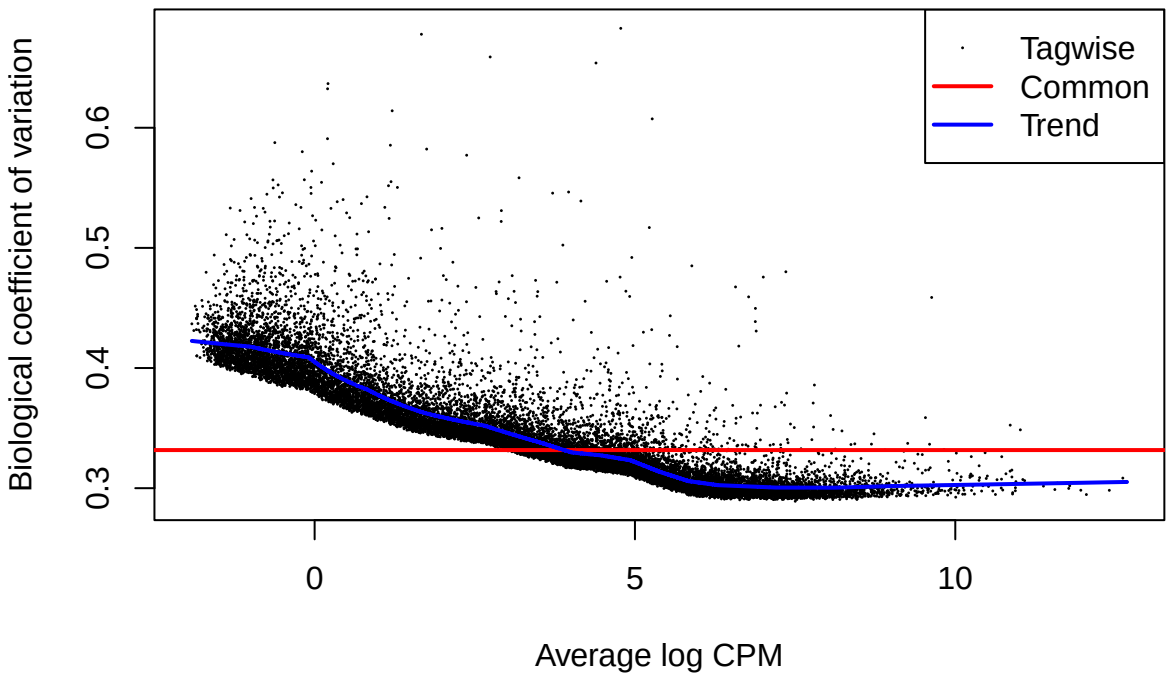
```
##    Mode   FALSE    TRUE
## logical   15672   20375
```

```
## MDS plot
#jpeg("plotMDS_mmusculus.jpg")
par(mar=c(6,6,6,6))
plotMDS(y,col=as.numeric(as.factor(infection)), pch=16, main="3 infected vs 3 controls")
par(xpd=T)
legend(par("usr")[2]*1.1,par("usr")[4]*0.8,sort(unique(infection)),
       pch=c(16),col=as.double(as.factor(sort(unique(infection)))))
```




```
#dev.off()
```

```
## Differential expression analysis
design <- model.matrix(~infection)
rownames(design) <- colnames(y)

y <- estimateDisp(y,design)
#jpeg("BCVplot_mmusculus.jpg")
plotBCV(y)
```




```
#dev.off()
#there is a trend!

fit_edgeR <- glmQLFit(y,design)
qlf_edgeR <- glmQLFTest(fit_edgeR,coef=2)
# coef=2 => infected vs control, see "design" object
# note: standard, the last column is considered as the contrast of interest
res_edgeR <- topTags(qlf_edgeR,n=nrow(qlf_edgeR$table))$table
```

```
## Add gene symbols
data_sorted_edgeR <- data[sort(data$ensembl_transcript_id,index.return=T)$ix,]
data_sorted_edgeR <- data_sorted_edgeR[duplicated(data_sorted_edgeR$ensembl_gene_id)==F,]

res_edgeR <- cbind(rownames(res_edgeR),res_edgeR)
colnames(res_edgeR)[1] <- "Ensembl_gene_id"

res_edgeR$diffexpressed <- "NO"
res_edgeR$diffexpressed[res_edgeR$logFC > 0 & res_edgeR$FDR < 0.05] <- "UP"
res_edgeR$diffexpressed[res_edgeR$logFC < 0 & res_edgeR$FDR < 0.05] <- "DOWN"

res_edgeR_sorted <- res_edgeR[sort(res_edgeR$Ensembl_gene_id,index.return=T)$ix,]
head(res_edgeR_sorted)
```

```
##                              Ensembl_gene_id      logFC   logCPM         F
## ENSMUSG00000000001.4   ENSMUSG00000000001.4  0.2742049 6.650000 0.5884430
```

```
## ENSMUSG00000000028.15 ENSMUSG00000000028.15 -0.3937051 4.530961 1.0743186
## ENSMUSG00000000037.16 ENSMUSG00000000037.16  0.7040886 0.522503 1.8979925
## ENSMUSG00000000056.7   ENSMUSG00000000056.7  0.1279611 4.663505 0.1152170
## ENSMUSG00000000058.6   ENSMUSG00000000058.6 -0.5199073 7.047578 2.0858873
## ENSMUSG00000000078.7   ENSMUSG00000000078.7  0.1639739 7.977682 0.2053453
##                           PValue       FDR diffexpressed
## ENSMUSG00000000001.4  0.4467346 0.8271373            NO
## ENSMUSG00000000028.15 0.3051047 0.7431571            NO
## ENSMUSG00000000037.16 0.1746180 0.6024115            NO
## ENSMUSG00000000056.7  0.7357444 0.9338949            NO
## ENSMUSG00000000058.6  0.1550858 0.5732253            NO
## ENSMUSG00000000078.7  0.6524583 0.9108295            NO
```

```
original <- res_edgeR$Ensembl_gene_id
#substr(original[1], 1, 18)
n <- 1
for (id in original){
  res_edgeR$Ensembl_gene_id[n] <- substr(id, 1, 18)
  n <- n + 1
}
head(res_edgeR$Ensembl_gene_id)
```

```
## [1] "ENSMUSG00000076612" "ENSMUSG00000100131" "ENSMUSG00000029417"
## [4] "ENSMUSG00000076613" "ENSMUSG00000094708" "ENSMUSG00000095937"
```

```
ids <- res_edgeR_sorted$Ensembl_gene_id
#substr(original[1], 1, 18)
n <- 1
for (id in ids){
  res_edgeR_sorted$Ensembl_gene_id[n] <- substr(id, 1, 18)
  n <- n + 1
}
#gsub
data_sorted_edgeR <- data_sorted_edgeR[data_sorted_edgeR$ensembl_gene_id%in%res_edgeR_sorted$Ensembl_ge
res_edgeR_sorted <- res_edgeR_sorted[res_edgeR_sorted$Ensembl_gene_id%in%data_sorted_edgeR$ensembl_gene

dim(res_edgeR_sorted) #19174
```

```
## [1] 20321     7
```

```
dim(data_sorted_edgeR) #19174
```

```
## [1] 20321     3
```

```
#which(res_edgeR_sorted$Ensembl_gene_id == data_sorted_edgeR$ensembl_transcript_id)

res_edgeR_sorted$Gene_symbol <- data_sorted_edgeR$external_gene_name



## Resort and save results
res_edgeR <- res_edgeR_sorted[sort(res_edgeR_sorted$PValue,index.return=T)$ix,]
head(res_edgeR[,c(1,7,2,5,6)],10) #geneID, symbol, logFC, pvalue, FDR
```

```
##                          Ensembl_gene_id diffexpressed      logFC       PValue
## ENSMUSG00000076612.8  ENSMUSG00000076612            UP   6.251235 3.580705e-17
## ENSMUSG00000100131.1  ENSMUSG00000100131          DOWN -12.409971 6.470052e-17
## ENSMUSG00000029417.9  ENSMUSG00000029417            UP   7.421845 6.322303e-16
## ENSMUSG00000076613.4  ENSMUSG00000076613            UP   5.750282 1.958567e-15
## ENSMUSG00000094708.1  ENSMUSG00000094708          DOWN  -9.590055 4.942463e-15
## ENSMUSG00000095937.1  ENSMUSG00000095937          DOWN  -9.514044 6.419399e-15
## ENSMUSG00000099875.1  ENSMUSG00000099875            UP   9.619127 1.212535e-14
## ENSMUSG00000031972.5  ENSMUSG00000031972            UP   8.506408 8.774228e-14
## ENSMUSG00000034855.13 ENSMUSG00000034855            UP   6.094653 2.401049e-13
## ENSMUSG00000042385.14 ENSMUSG00000042385            UP   6.964233 2.638372e-13
##                                FDR
## ENSMUSG00000076612.8  6.591366e-13
## ENSMUSG00000100131.1  6.591366e-13
## ENSMUSG00000029417.9  4.293898e-12
## ENSMUSG00000076613.4  9.976453e-12
## ENSMUSG00000094708.1  2.014054e-11
## ENSMUSG00000095937.1  2.179921e-11
## ENSMUSG00000099875.1  3.529343e-11
## ENSMUSG00000031972.5  2.234686e-10
## ENSMUSG00000034855.13 5.375683e-10
## ENSMUSG00000042385.14 5.375683e-10
```

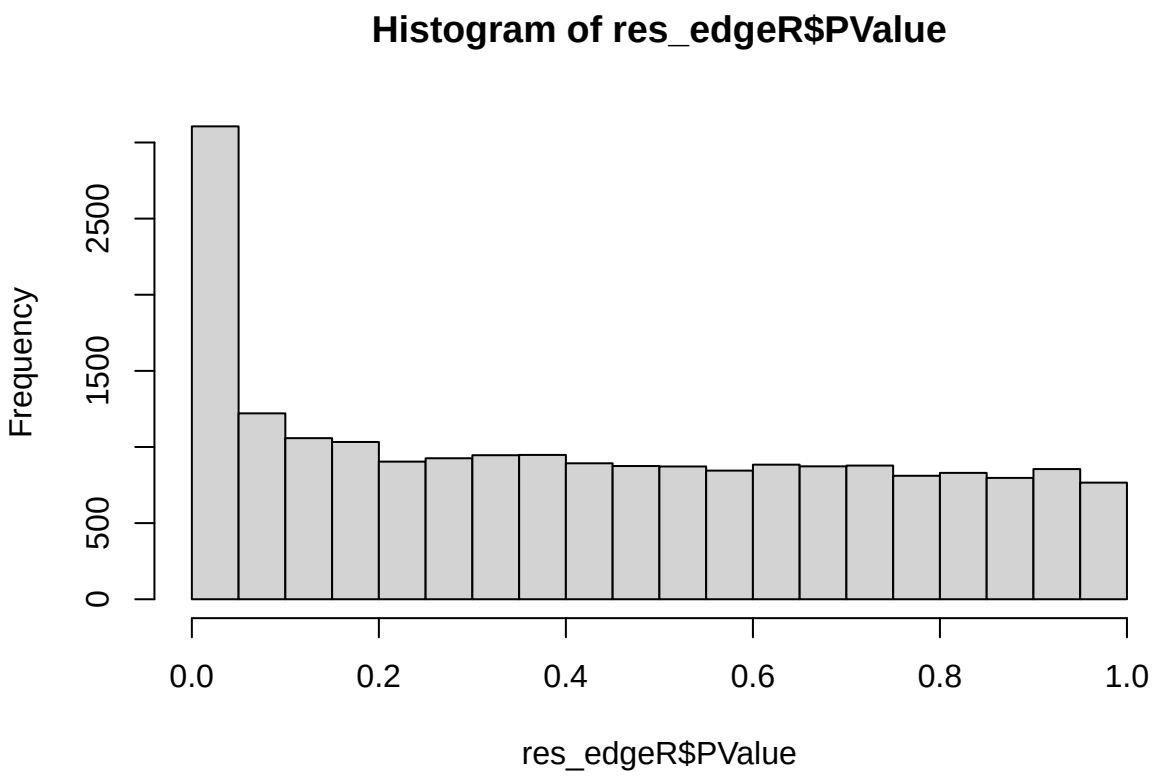
```
write.table(res_edgeR,file="res_edgeR_mmusculus.txt",col.names=T,row.names=T,sep="\t",quote=F)
# top 1000 loci, also contain non-significant loci (filtered out in next step)
res_edgeR_sign <- res_edgeR[res_edgeR$FDR<0.05,]
dim(res_edgeR_sign)
```

```
## [1] 1329    8
```

```
# last column of res (ncol(res)) contains FDRs => filtering at 5% level


## MA plot
jpeg("res_edgeR_mmusuculus_MA.png")
## MA-plot
with(res_edgeR,plot(logCPM,logFC,pch=16,cex=0.2))
# MAplot: all data points
with(res_edgeR,points(logCPM[FDR<0.05],logFC[FDR<0.05],pch=16,col="red",cex=0.6))
# MA-plot: significant loci
abline(0,0)
# X-axis
dev.off()
```

```
## pdf
##   2
```

```
## Pvalue distribution
#jpeg("res_edgeR_mmusculus_histogram.jpg")
hist(res_edgeR$PValue)
```




```
#dev.off()


## Plot boxplots for top 20 loci (first make the folder where you want to put them!)
setwd("./RNAseq_output/Boxplots/")
counts_k <- txi.kallisto$counts[keep,]
for (i in 1:20){
  counts_part <- as.numeric(cpm(y)[rownames(counts_k)==rownames(res_edgeR)[i],])
  dat_boxplot <- data.frame(counts=counts_part,group=infection)
  jpeg(paste(i,"_",rownames(res_edgeR)[i],".jpg",sep=""))
  if (res_edgeR$Gene_symbol[i]!=""){
    boxplot(counts~group,dat_boxplot,main=paste(rownames(res_edgeR)[i], " (",res_edgeR$Gene_symbol[i],"
  } else {
    boxplot(counts~group,dat_boxplot,main=paste(rownames(res_edgeR)[i], " (NA)",sep=""))
  }
  dev.off()
}
```

```
RNAseq_DEgenes <- res_edgeR_sign$Gene_symbol
head(RNAseq_DEgenes)
```

```
## [1] "Thy1"      "Hmgb1-ps8" "Rhbdd3"    "Uhrf1bp1"  "Gm17235"   "Hspe1-ps3"
```

```
length(RNAseq_DEgenes)
```

```
## [1] 1329
```

```
length(which(res_edgeR$diffexpressed=="UP")) #99
```

```
## [1] 1230
```

```
length(which(res_edgeR$diffexpressed=="DOWN")) #1230
```

```
## [1] 99
```

```
#List all down and upregulated genes for each dataset
Array1_upregulated <- unique(annotation_MA[annotation_MA$ID %in% rownames(LIMMAout_annot[LIMMAout_annot$
length(Array1_upregulated) #102
```

```
## [1] 102
```

```
Array1_downregulated <- unique(annotation_MA[annotation_MA$ID %in% rownames(LIMMAout_annot[LIMMAout_anno
length(Array1_downregulated)      #357
```

```
## [1] 357
```

```
#Microarray2
Array2_up <- LIMMAout_sorted2$gene[LIMMAout_sorted2$logFC > 1 & LIMMAout_sorted2$adj.P.Val <= 0.05]
Array2_upregulated <- NULL
for (gene in Array2_up){
  n <- which(gene == gene_list)
  Array2_upregulated <- c(Array2_upregulated, unique(annotLookup$external_gene_name)[n])
}

Array2_down <- LIMMAout_sorted2$gene[LIMMAout_sorted2$logFC < -1 & LIMMAout_sorted2$adj.P.Val <= 0.05]
Array2_downregulated <- NULL
for (gene in Array2_down){
  n <- which(gene == gene_list)
  Array2_downregulated <- c(Array2_downregulated, unique(annotLookup$external_gene_name)[n])
}
head(Array2_downregulated)
```

```
## [1] "Tex11" "Per3"  "Per3"  "Nr1d2" "Sspn"  "Ces1f"
```

```
RNA_upregulated <- res_edgeR$Gene_symbol[res_edgeR$diffexpressed=="UP"]
RNA_downregulated <- res_edgeR$Gene_symbol[res_edgeR$diffexpressed=="DOWN"]
```

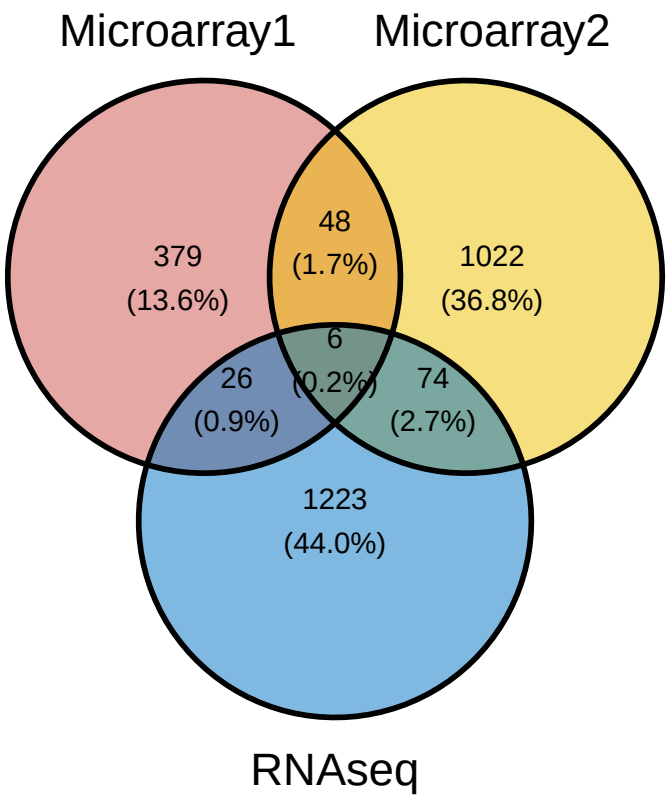
```
library(ggvenn)
```

```
## Loading required package: dplyr
```

```
##
## Attaching package: 'dplyr'

## The following object is masked from 'package:biomaRt':
##
##     select

## The following object is masked from 'package:lumi':
##
##     combine

## The following object is masked from 'package:methylumi':
##
##     combine

## The following object is masked from 'package:minfi':
##
##     combine

## The following objects are masked from 'package:GenomicRanges':
##
##     intersect, setdiff, union

## The following object is masked from 'package:matrixStats':
##
##     count

## The following object is masked from 'package:oligo':
##
##     summarize

## The following objects are masked from 'package:Biostrings':
##
##     collapse, intersect, setdiff, setequal, union

## The following object is masked from 'package:GenomeInfoDb':
##
##     intersect

## The following object is masked from 'package:XVector':
##
##     slice

## The following object is masked from 'package:AnnotationDbi':
##
##     select

## The following objects are masked from 'package:IRanges':
##
##     collapse, desc, intersect, setdiff, slice, union
```

```
## The following objects are masked from 'package:S4Vectors':
##
##     first, intersect, rename, setdiff, setequal, union

## The following object is masked from 'package:Biobase':
##
##     combine

## The following objects are masked from 'package:BiocGenerics':
##
##     combine, intersect, setdiff, union

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union

## Loading required package: grid

##
## Attaching package: 'grid'

## The following object is masked from 'package:Biostrings':
##
##     pattern
```

```
overlap <-list('Microarray1'= DEgenes_symbols1,'Microarray2'=DEgene_symbols2, 'RNAseq'=RNAseq_DEgenes)

#Create venn diagram and display all the sets
ggvenn(overlap, fill_color = c("#CD534CFF", "#EFC000FF", "#0073C2FF")
)
```



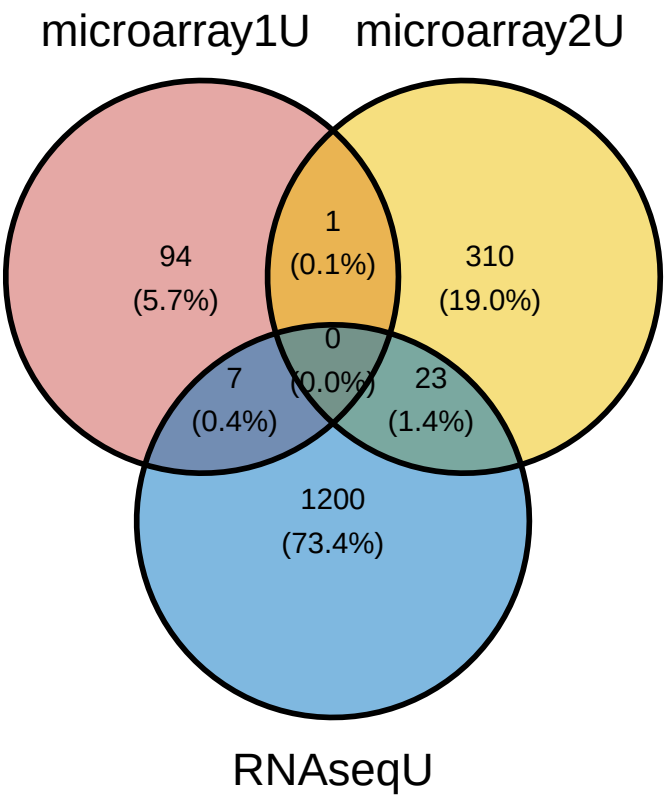

```
common_DEgenes <- Reduce(intersect, list(DEgenes_symbols1,DEgene_symbols2, RNAseq_DEgenes))
array1_RNA <- Reduce(intersect, list(DEgenes_symbols1, RNAseq_DEgenes))
array1_RNA
```

```
##  [1] "Il1r2"          "Rpa3"           "Kansl1l"        "Slc25a5"
##  [5] "Fuca2"          "Slfn4"          "Zbtb1"          "Commd6"
##  [9] "C730034F03Rik" "Prdx1"          "Samsn1"         "Tnfsf9"
## [13] "Gca"            "Lpcat4"         "Il1b"           "Slc16a1"
## [17] "Pitx2"          "Lef1"           "Ssu72"          "Ndufb6"
## [21] "Cux1"           "Gpnmb"          "Tnip3"          "Ccnd2"
## [25] "Cd79a"          "Pde8a"          "Swap70"         "Zpr1"
## [29] "Nt5e"           "Bbs4"           "Dynlt3"         "Gla"
```

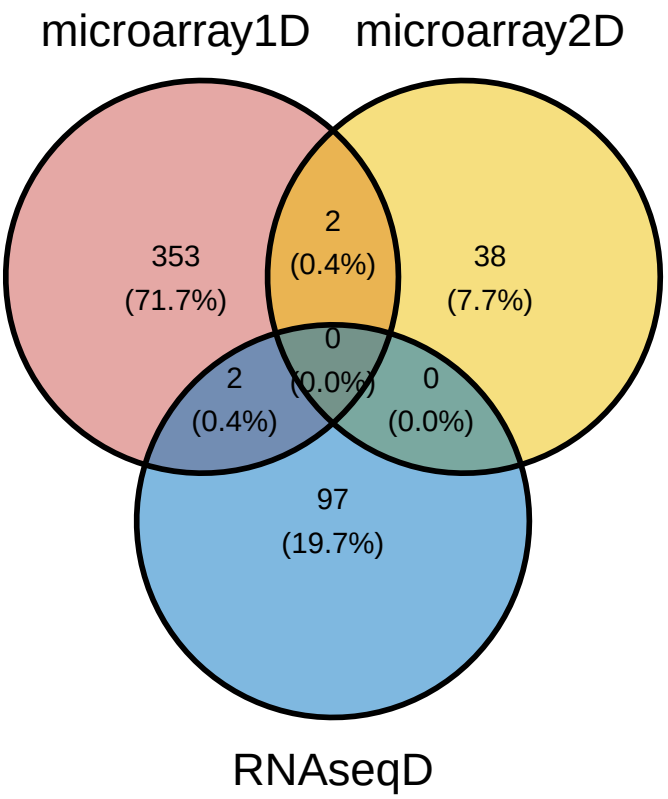
```
head(common_DEgenes)
```

```
## [1] "Il1r2"  "Samsn1" "Il1b"   "Ccnd2"  "Swap70" "Gla"
```

```
UP <- list("microarray1U"=Array1_upregulated, "microarray2U"=Array2_upregulated, "RNAseqU"=RNA_upregula
DOWN <- list('microarray1D'= Array1_downregulated, "microarray2D"=Array2_downregulated, "RNAseqD"=RNA_d

# upregulated genes for all 3 datasets
ggvenn(UP, fill_color = c("#CD534CFF", "#EFC000FF", "#0073C2FF"))
```




```
common_upreg <- Reduce(intersect, list(Array1_upregulated, Array2_upregulated, RNA_upregulated))

# downregulated genes for all 3 datasets
ggvenn(DOWN, fill_color = c("#CD534CFF", "#EFC000FF", "#0073C2FF"))
```




```
common_downreg <- Reduce(intersect, list(Array1_downregulated, Array2_downregulated, RNA_downregulated))
```