

microarray2

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12/8/2021

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 dimensionality of the intensity value matrix.

```
id <- "E-GEOD-64750"
exonCEls <- list.celfiles("C:/Users/tobia/Downloads/Array2")
data.raw_2 <- read.celfiles(paste(rep("C:/Users/tobia/Downloads/Array2/", length(exonCEls)), exonCEls, sep = ""))

## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579245_jac013-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579246_jac021-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579247_jac044-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579248_jac045-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579249_jac046-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579250_jac007-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579251_jac008-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579252_jac036-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579253_jac047-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579254_jac014-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579255_jac024-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579256_jac025-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579257_jac011-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579258_jac012-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579259_jac037-430v2.CEL
## Reading in : C:/Users/tobia/Downloads/Array2/GSM1579260_jac048-430v2.CEL

dim(exprs(data.raw_2))

## [1] 1004004      16

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

```

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("C:/Users/tobia/Downloads/Array2/E-GEO-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 uninfected mice
## 27                                     Gene expression data from lungs of uninfected mice

```

```

## 28                               Gene expression data from lungs of uninfected 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 uninfected mice
## 34                               Gene expression data from lungs of uninfected mice
## 35                               Gene expression data from lungs of uninfected mice
## 36                               Gene expression data from lungs of uninfected mice
## 37                               Gene expression data from lungs of uninfected 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 first lines of output from the object
#head(exprs(MouseExp_pheno2))
#pData(MouseExp_pheno2) #source name, comment sample description, sample source name, sample title, ch

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

## [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"

filtered <- data.raw_2[,filter]
filtered

## 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      16 samples

## [1] 1004004      16

```

```

head(exprs(filtered))

##   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(filtered,outdir="./rawCD",force=T)

## The report will be written into directory './rawCD'.

## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

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## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

```

```

## name(s): subscripts, group.number, group.value

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

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## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

```

```

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, gridsize = nbin, bandwidth = bandwidth):
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

arrayQualityMetrics(filtered,outdir=".~/rawlog",force=T,do.logtransform=T)

## The report will be written into directory './rawlog'.

## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

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## name(s): subscripts, group.number, group.value

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## name(s): subscripts, group.number, group.value

## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value
miceRMA <- oligo::rma(filtered, background=T)

## Background correcting
## Normalizing
## Calculating Expression
miceRMA

## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 45101 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, force=TRUE)

## The report will be written into directory 'arrayQualityMetrics' report for miceRMA'.

## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

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## name(s): subscripts, group.number, group.value

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## Warning in svgStyleAttributes(style, svgdev): Removing non-SVG style attribute
## name(s): subscripts, group.number, group.value

## 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 control"))
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, "infected"))

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

fit2 <- contrasts.fit(fit,cont.matrix)
fit2 <- eBayes(fit2)
fit2

## 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

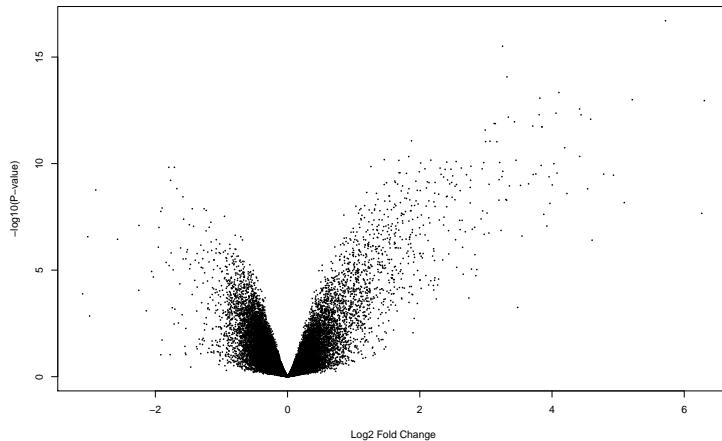
```

```

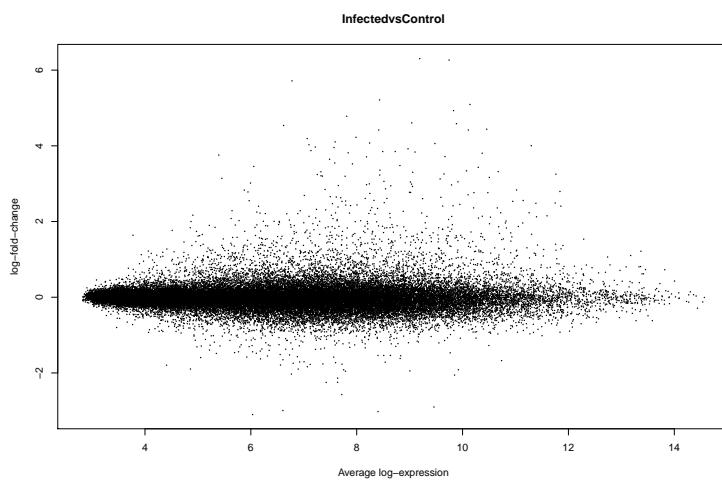
##
## $var.prior
## [1] 61.79253
##
## $proportion
## [1] 0.01
##
## $s2.post
##   1415670_at   1415671_at   1415672_at   1415673_at 1415674_a_at
##   0.08665770   0.03981860   0.01606514   0.08671723   0.02171072
## 45096 more elements ...
##
## $t
##           Contrasts
##           InfectedvsControl
##   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)
```



```
limma::plotMA(fit2)
```



```
# DE results
LIMMAout <- topTable(fit2, adjust="BH", number=nrow(exprs(miceRMA)))
#head(LIMMAout)

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

##          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(LIMMAout)), 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(LIMMAout)), 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

#mean expression of control/C57BL6
rowMeans(exprs(miceRMA)[rownames(exprs(miceRMA))%in%rownames(head(LIMMAout)),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

#mean expression of infected/C57BL6
rowMeans(exprs(miceRMA)[rownames(exprs(miceRMA))%in%rownames(head(LIMMAout)),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

#Adjustments on p values using Benjamini-Hochberg
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"

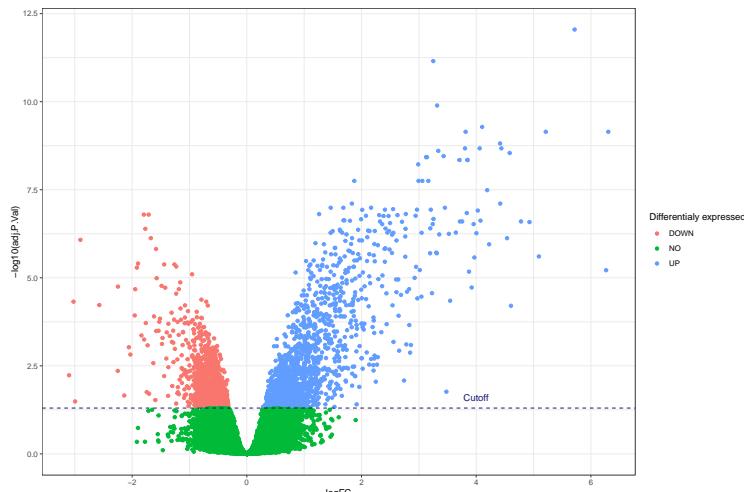
#No adjustments on pvalues
LIMMAout$diffexpressed_no_BH <- "NO"
LIMMAout$diffexpressed_no_BH[LIMMAout$logFC > 0 & LIMMAout$P.Value < 0.05] <- "UP"
LIMMAout$diffexpressed_no_BH[LIMMAout$logFC < 0 & LIMMAout$P.Value < 0.05] <- "DOWN"

C57_down <- LIMMAout[LIMMAout$diffexpressed_no_BH=="DOWN", ]
C57_up <- LIMMAout[LIMMAout$diffexpressed_no_BH=="UP", ]

a <- subset(LIMMAout, LIMMAout$diffexpressed_no_BH=="DOWN")

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")+
  #ggtitle("Differentiall (unadjusted P-value)") +
  labs(colour = "Differentially expressed")

```

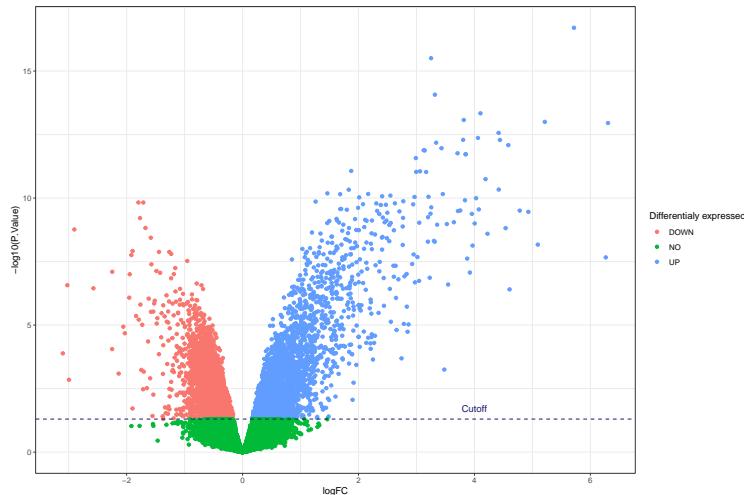


```
#theme(plot.title = element_text(hjust = 0.5, face = "bold.italic"))
```

```

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")+
  #ggtitle("Differentiall (unadjusted P-value)") +
  labs(colour = "Differentially expressed")

```



```

length(which(LIMMAout$diffexpressed=="UP"))

## [1] 1324

length(which(LIMMAout$diffexpressed=="DOWN"))

## [1] 1039

length(which(LIMMAout$diffexpressed_no_BH=="UP"))

## [1] 3114

length(which(LIMMAout$diffexpressed_no_BH=="DOWN"))

## [1] 3867

## Load annotation and sort alphabetically on probe name
annotation_MA <- read.table("C:/Users/tobia/Downloads/Array2/A-AFFY-45.adf.txt", header=T, sep="\t", skip=1)
print(head(annotation_MA))

##   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_MA <- annotation_MA[sort(annotation_MA$Composite.Element.Name, index.return=T)$ix,]

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

## [1] 45101    16
dim(LIMMAout)

## [1] 45101     8
## Double check => "Assumption is the mother of all fuck up's ;)"
sum(annotation_MA$Composite.Element.Name==sort(rownames(LIMMAout)))

## [1] 45101
## Sort LIMMA output alphabetically on probe name
LIMMAout_sorted <- LIMMAout[sort(rownames(LIMMAout), index.return=T)$ix,]
#LIMMAout_sorted

## Add gene names to LIMMA output
LIMMAout_sorted$gene <- annotation_MA$Composite.Element.Database.Entry.ensembl.
LIMMAout_annot <- LIMMAout_sorted[sort(LIMMAout_sorted$adj.P.Val, index.return=T)$ix,]

#sort by adjusted p value from most significant to least
LIMMAout_sorted2 <- LIMMAout_sorted[order(LIMMAout_sorted$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_annot)

##          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_annot[LIMMAout_annot$gene=="ENSMUSG00000022548",]

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

#extract DE genes
DEgenes <- LIMMAout_sorted2$gene[LIMMAout_sorted2$adj.P.Val <= 0.05]
DEgenes <- DEgenes[DEgenes != ""]

head(DEgenes)

## [1] "ENSMUSG00000025746" "ENSMUSG00000045303" "ENSMUSG00000045932"
## [4] "ENSMUSG00000051925" "ENSMUSG00000034855" "ENSMUSG00000029419"

```