BB512/BB612 - Week VI

```
suppressPackageStartupMessages(library(Biobase))
suppressPackageStartupMessages(library(bladderbatch))
suppressPackageStartupMessages(library(sva))
```

Data

We are going to use data from the paper Evaluating gene expression in C57BL/6J and DBA/2J mouse striatum using RNA-Seq and microarrays. that is a comparative RNA-seq analysis of different mouse strains.

```
con <- url("http://bowtie-bio.sourceforge.net/recount/ExpressionSets/bottomly_eset.RData")
load(file = con)
close(con)

pdata <- pData(bottomly.eset)
edata <- as.matrix(exprs(bottomly.eset))
fdata <- fData(bottomly.eset)
```

Transform the expression data and remove lowly expressed genes:

```
edata <- log2(as.matrix(edata) + 1)
edata <- edata[rowMeans(edata) > 10, ]
```

```
head(pdata)
```

```
##           sample.id num.tech.reps   strain experiment.number lane.number
## SRX033480 SRX033480             1 C57BL/6J                 6           1
## SRX033488 SRX033488             1 C57BL/6J                 7           1
## SRX033481 SRX033481             1 C57BL/6J                 6           2
## SRX033489 SRX033489             1 C57BL/6J                 7           2
## SRX033482 SRX033482             1 C57BL/6J                 6           3
## SRX033490 SRX033490             1 C57BL/6J                 7           3
```

```
table(pdata$strain)
```

```
##
## C57BL/6J   DBA/2J
##       10       11
```

Calculate p-values parametrically

t-test

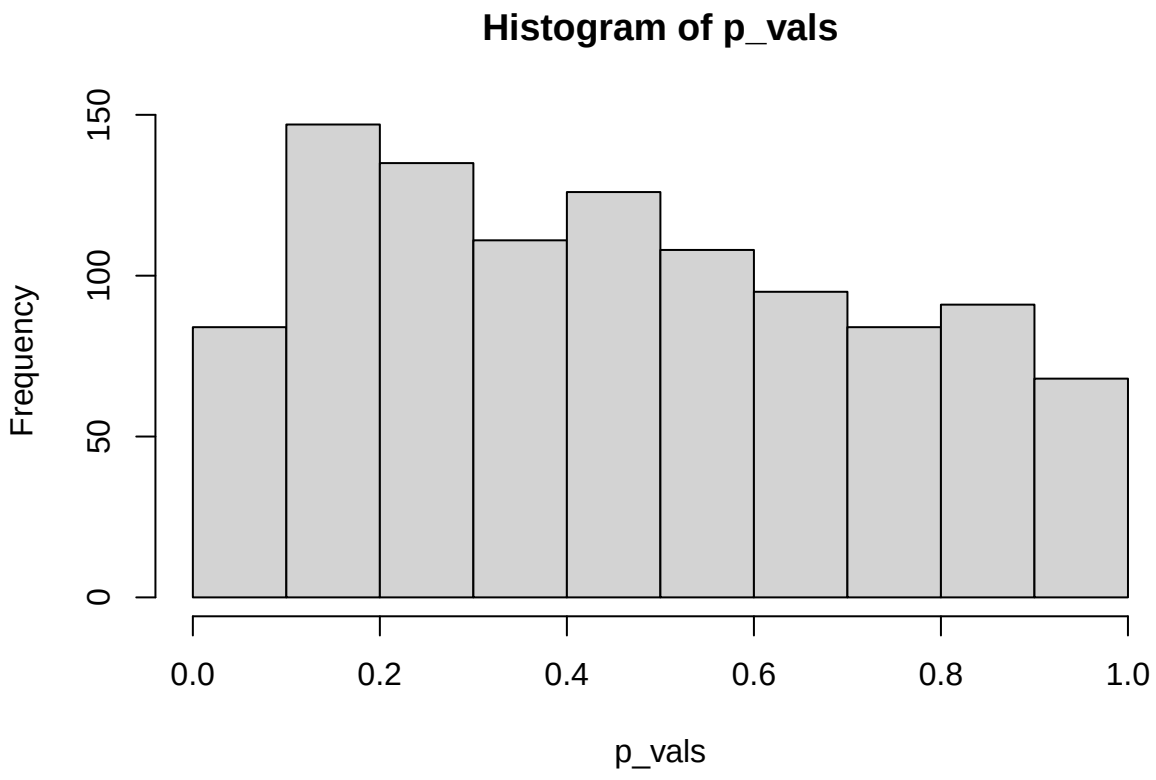
```
idxA <- which(colnames(edata) %in% pdata$sample.id[pdata$strain == "C57BL/6J"])
idxB <- which(colnames(edata) %in% pdata$sample.id[pdata$strain == "DBA/2J"])
raw_p_vals <- apply(edata, 1, function(x) t.test(x[idxA], x[idxB])$p.value)
p_vals <- sort(raw_p_vals)
head(p_vals, 10)
```

```
## ENSMUSG00000033585 ENSMUSG00000070003 ENSMUSG00000022212 ENSMUSG00000044708
##         0.00019311         0.00348277         0.00573420         0.00738418
## ENSMUSG00000024883 ENSMUSG00000036473 ENSMUSG00000038020 ENSMUSG00000074045
##         0.00927920         0.01023011         0.01119852         0.01435736
## ENSMUSG00000032340 ENSMUSG00000041297
##         0.01560463         0.01826414
```

```
p_adj_vals <- p.adjust(p_vals, method = "fdr")
head(p_adj_vals, 10)
```

```
## ENSMUSG00000033585 ENSMUSG00000070003 ENSMUSG00000022212 ENSMUSG00000044708
##            0.20257            0.84267            0.84267            0.84267
## ENSMUSG00000024883 ENSMUSG00000036473 ENSMUSG00000038020 ENSMUSG00000074045
##            0.84267            0.84267            0.84267            0.84267
## ENSMUSG00000032340 ENSMUSG00000041297
##            0.84267            0.84267
```

```
hist(p_vals)
```



Adjusting for variables with `edge`

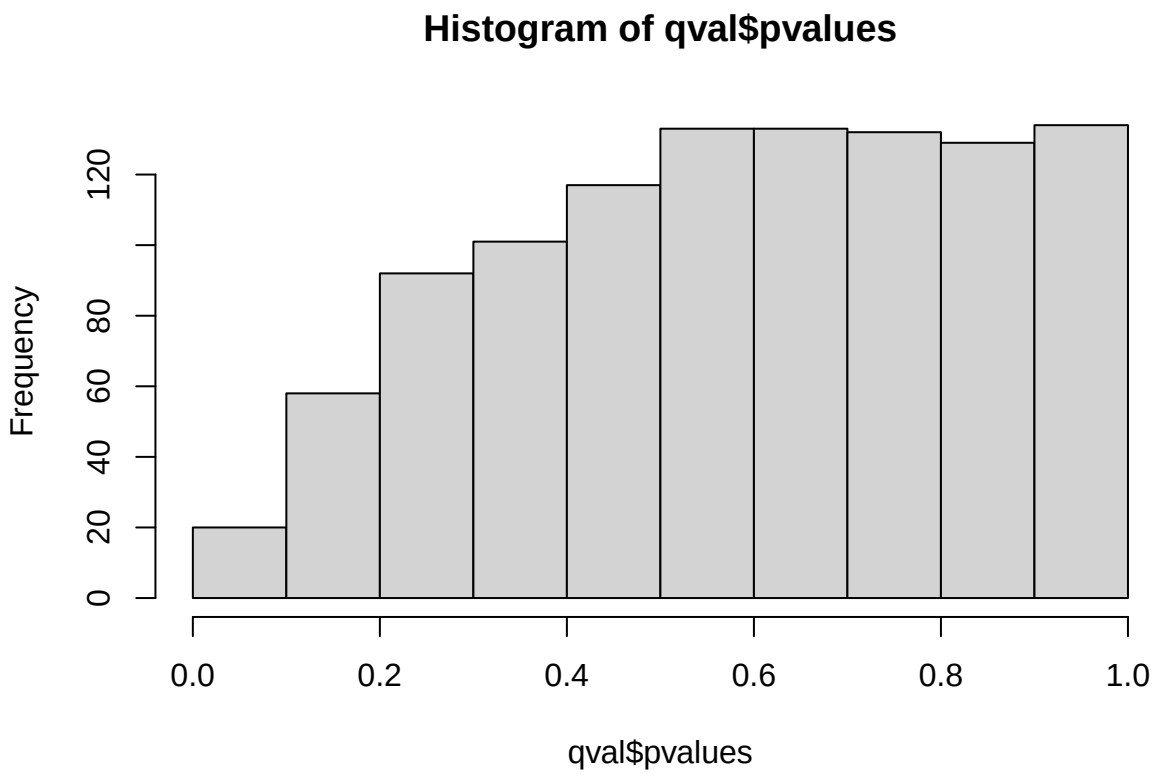
```
devtools::install_github("jdstorey/edge")
```

```
## Using github PAT from envvar GITHUB_PAT
```

```
## Skipping install of 'edge' from a github remote, the SHA1 (5f973def) has not changed since last insta
##   Use `force = TRUE` to force installation
```

```
library(edge)

edge_study <- build_study(edata, grp = pdata$strain,
                          adj.var = as.factor(pdata$lane.number))
de_obj <- lrt(edge_study)
qval <- qvalueObj(de_obj)
hist(qval$pvalues)
```



Using moderated statistics with limma

```
library(limma)
```

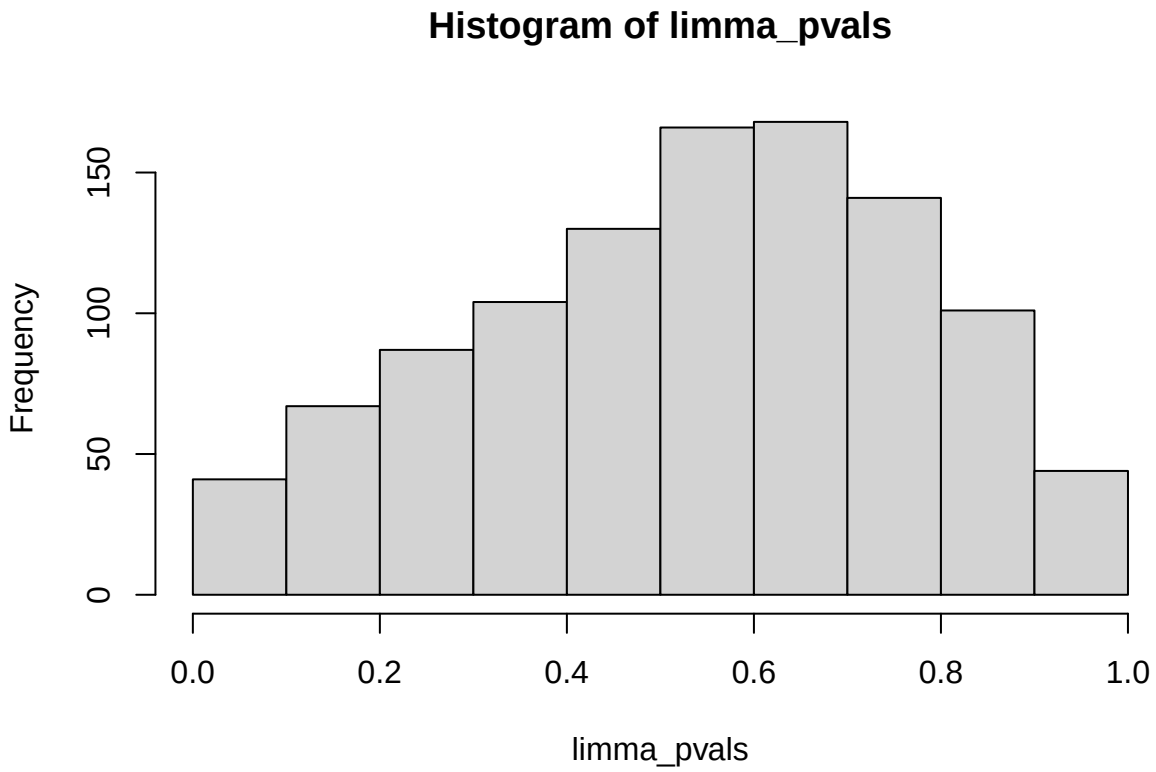
```
##
## Attaching package: 'limma'
```

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

```
mod <- model.matrix(~ pdata$strain + pdata$lane.number)
fit_limma <- lmFit(edata, mod)
ebayes_limma <- eBayes(fit_limma)
limma_pvals <- topTable(ebayes_limma, number = Inf)$P.Value
```

```
## Removing intercept from test coefficients
```

```r
hist(limma_pvals)
```



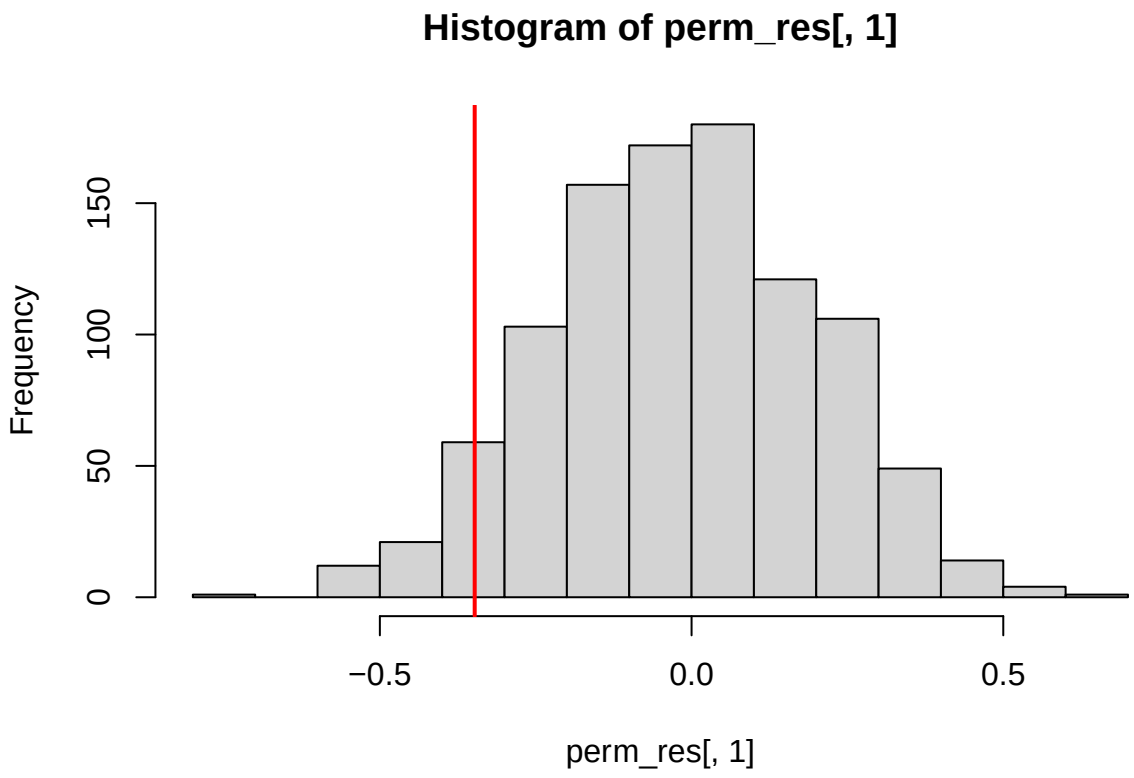
Calculating empirical permutation p-values

```r
idxA <- which(colnames(edata) %in% pdata$sample.id[pdata$strain == "C57BL/6J"])
idxB <- which(colnames(edata) %in% pdata$sample.id[pdata$strain == "DBA/2J"])

actual_diffs <- apply(edata, 1, function(x) mean(x[idxA]) - mean(x[idxB]))

B <- 1000
perm_res <- c()
set.seed(123)
for (i in seq_len(B)) {
    cur_idxA <- sample(seq_len(ncol(edata)), 10)
    cur_idxB <- setdiff(seq_len(ncol(edata)), cur_idxA)
    cur_diffs <- apply(edata, 1, function(x) mean(x[cur_idxA]) - mean(x[cur_idxB]))
    perm_res <- rbind(perm_res, cur_diffs)
}

hist(perm_res[, 1])
abline(v = actual_diffs[1], col = "red", lwd = 2)
```

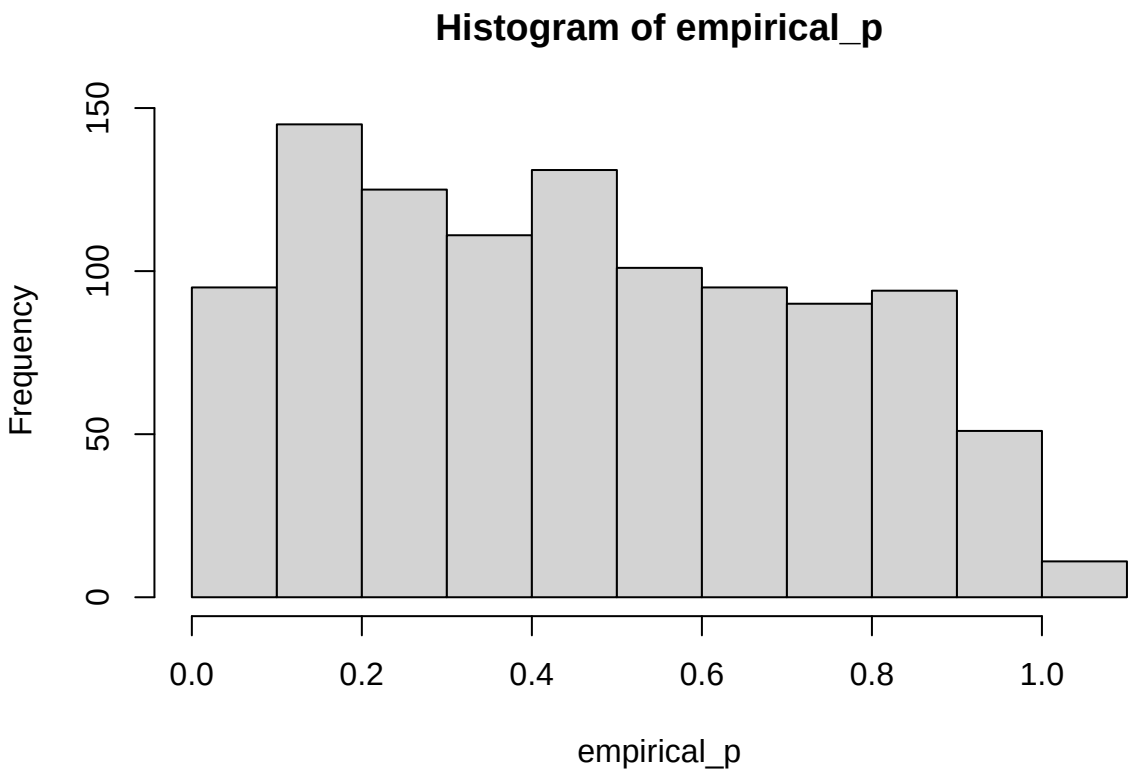



```
# empirical p value
sum(perm_res[, 1] <= actual_diffs[1]) / B
```

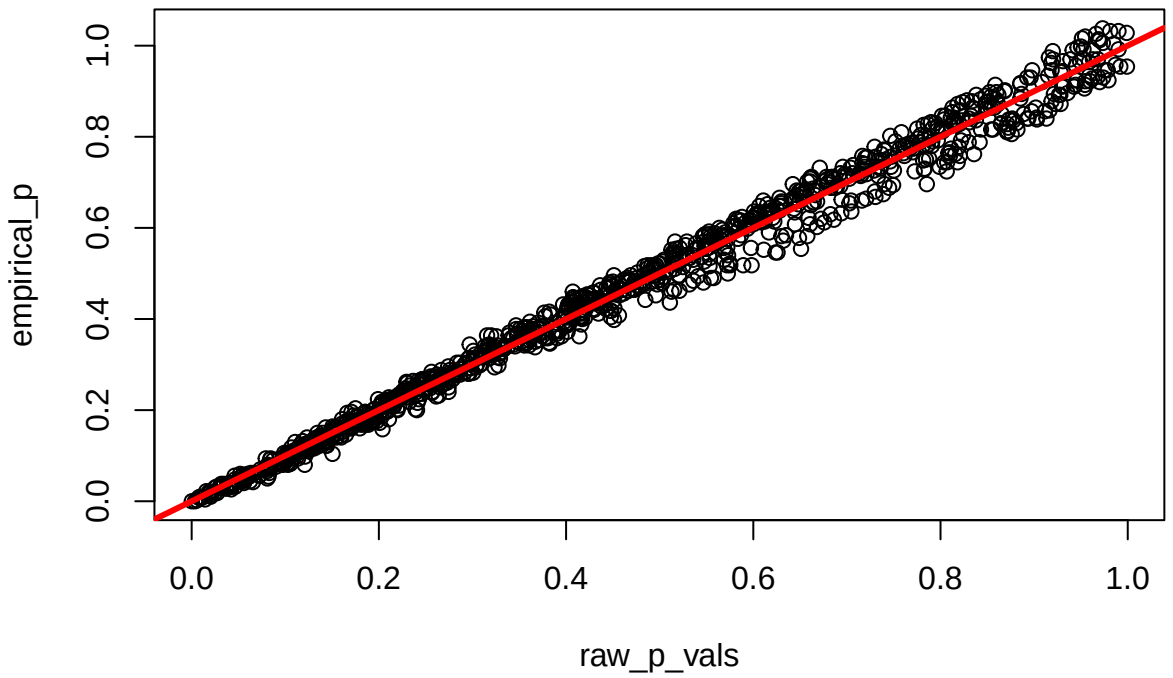
```
## [1] 0.057
```

```
empirical_p <- c()
for (j in 1:ncol(perm_res)) {
    if (actual_diffs[j] < 0) {
        empirical_p <- c(empirical_p, 2 * sum(perm_res[, j] <= actual_diffs[j]) / B)
    } else {
        empirical_p <- c(empirical_p, 2 *sum(perm_res[, j] >= actual_diffs[j]) / B)
    }
}

hist(empirical_p)
```




```
plot(raw_p_vals, empirical_p)
abline(a = 0, b = 1, col = "red", lwd = 3)
```


empirical_p
0.0
0.2
0.4
0.6
0.8
1.0
raw_p_vals
0.0
0.2
0.4
0.6
0.8
1.0