Reanalysis Johnson2007 - figures and code

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The `sessionInfo()` is provided at the end of the document.

```
rm(list = ls())

library(GGally)
## Loading required package: ggplot2
## Registered S3 method overwritten by 'GGally':
##   method from
##   +.gg   ggplot2
library(ggplot2)
library(limma)
library(sva)
## Loading required package: mgcv
## Loading required package: nlme
## This is mgcv 1.8-33. For overview type 'help("mgcv-package")'.
## Loading required package: genefilter
## Loading required package: BiocParallel
library(qvalue)
library(randRotation)
```

File from https://github.com/ous-uio-bioinfo-core/batch-adjust-warning-figures/tree/master/reanalysis/Johnson2007/data See also Nygaard, V., Rodland, E. A. & Hovig, E. Methods that remove batch effects while retaining group differences may lead to exaggerated confidence in downstream analyses. Biostatistics kxv027 (2015). doi:10.1093/biostatistics/kxv027

```
edata <- read.table(file = "dataExample2.txt", header = TRUE,
                    sep = "\t", dec = ".", stringsAsFactors = FALSE)
```

File from https://github.com/ous-uio-bioinfo-core/batch-adjust-warning-figures/tree/master/reanalysis/Johnson2007/data See also Nygaard, V., Rodland, E. A. & Hovig, E. Methods that remove batch effects while retaining group differences may lead to exaggerated confidence in downstream analyses. Biostatistics kxv027 (2015). doi:10.1093/biostatistics/kxv027

```
pdata <- read.table(file = "sampleInfoExample2.txt", header = TRUE,
                    sep = "\t", dec = ".", stringsAsFactors = TRUE,
                    row.names = 1)

samps <- which(pdata$Type != "WT")

pdata <- pdata[samps,]
edata <- edata[,samps]
pdata$Type <- droplevels(pdata$Type)
pdata$Batch <- as.factor(pdata$Batch)
```

```
all.equal(rownames(pdata), colnames(edata))
## [1] TRUE



# flooring to 1
edata[edata<1] <- 1
# take out data with too many low/missing values.
negativeprobesfilter <- rowSums(edata>1) >= (0.9*ncol(edata))
edata <- edata[negativeprobesfilter,]
# quantilenormalize
edata.quan <- normalizeBetweenArrays(log2(edata), method="quantile")
```

```
# for debugging
debug = FALSE
if(debug) edata <- edata[1:1000,]
```

```
#### ComBat - "p ComBat" values

mod.com <- model.matrix(~Type, pdata)
edata.com <- ComBat(edata.quan,
                    batch = pdata$Batch,
                    mod = mod.com)
## Found3batches
## Adjusting for1covariate(s) or covariate level(s)
## Standardizing Data across genes
## Fitting L/S model and finding priors
## Finding parametric adjustments
## Adjusting the Data


mod.fit <- model.matrix(~Type, pdata)
fit1 <- lmFit(edata.com, design = mod.fit)
fit1 <- eBayes(fit1)

ps.com  <- topTable(fit1, number = Inf, sort.by = "none")$P.Value
## Removing intercept from test coefficients
fdr.com <- topTable(fit1, number = Inf, sort.by = "none")$adj.P.Val
## Removing intercept from test coefficients

sum(fdr.com<0.05)
## [1] 649
sum(qvalue(ps.com)$qvalues < 0.05)
## [1] 814
```

```
#### limma batch as covariate - "p Limma (+batch)" values

mod.fit <- model.matrix(~Type + Batch, pdata)
fit2 <- lmFit(edata.quan, design = mod.fit)
fit2 <- eBayes(fit2)
```

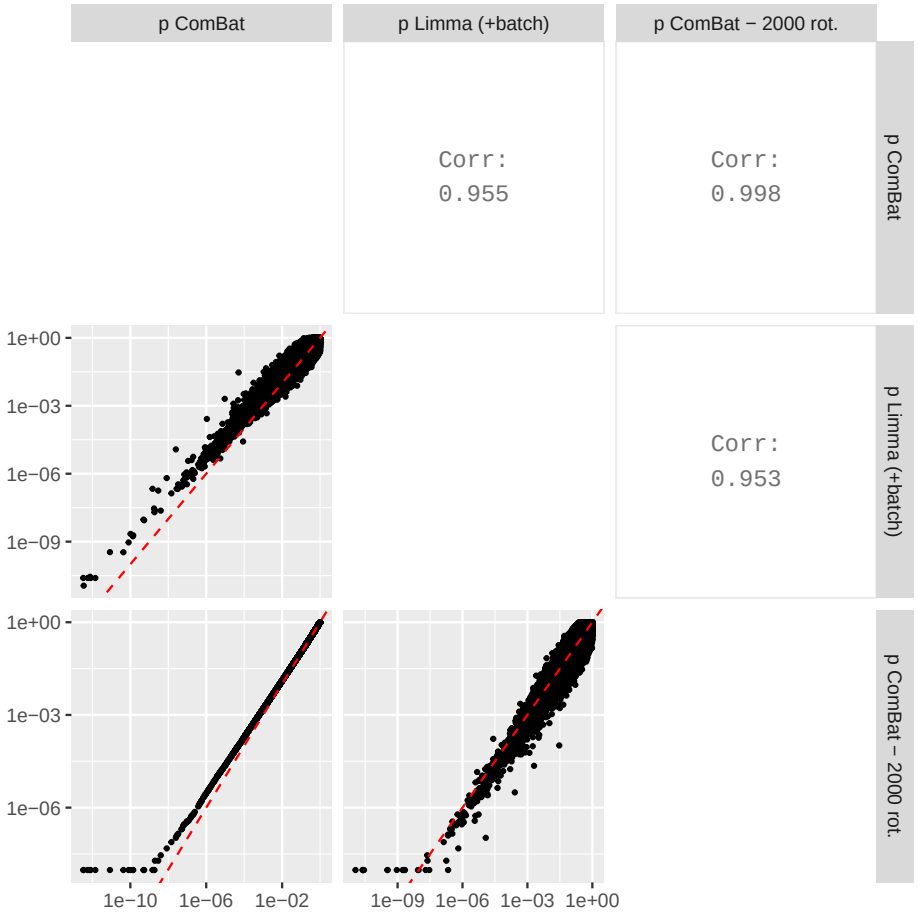
```
ps.lim  <- topTable(fit2, coef = 2, number = Inf, sort.by = "none")$P.Value
fdr.lim <- topTable(fit2, coef = 2, number = Inf, sort.by = "none")$adj.P.Val

sum(fdr.lim<0.05)
## [1] 271
sum(qvalue(ps.lim)$qvalues < 0.05)
## [1] 392
```

```
#### ComBat with random rotations - "p ComBat - 2000 rot." values

mod.fit <- model.matrix(~Type, pdata)

rr1 <- initBatchRandrot(edata.quan, mod.fit, 2, pdata$Batch)
## Initialising batch "1"
## Initialising batch "2"
## Initialising batch "3"

statistic <- function(Y, batch, mod){
    edata.com <- sva::ComBat(Y,
                             batch = batch,
                             mod = mod, mean.only = FALSE)

    fit1 <- limma::lmFit(edata.com, design = mod)
    fit1 <- limma::eBayes(fit1)

    abs(limma::topTable(fit1, number = Inf, sort.by = "none")$t)
}
```

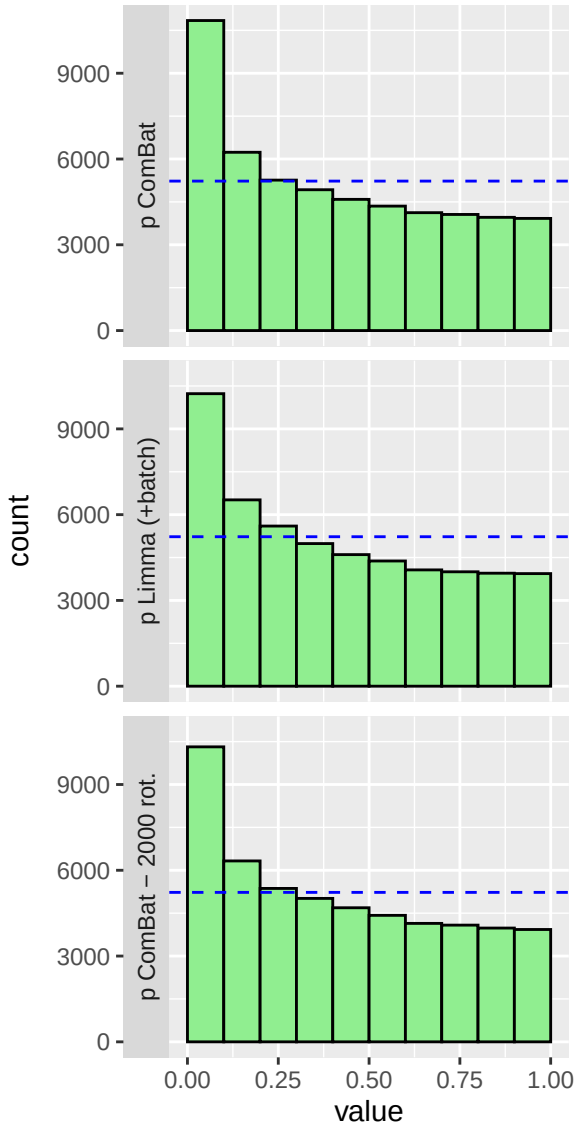
```
rs1 <- rotateStat(rr1, R = 2000, statistic = statistic, pdata$Batch,
                  mod.fit, parallel = TRUE)
```

```
ps.rot <- pFdr(rs1)
fdr.rot <- p.adjust(ps.rot, "BH")

sum(fdr.rot<0.05)
## [1] 431
sum(qvalue(ps.rot)$qvalues < 0.05)
## [1] 533

ps <- cbind(ps.com, ps.lim, ps.rot = ps.rot[,1])
colnames(ps) <- c("p ComBat", "p Limma (+batch)", "p ComBat - 2000 rot.")

apply(ps, 2, function(i)sum(qvalue::qvalue(i)$qvalues < 0.05))
##             p ComBat     p Limma (+batch) p ComBat - 2000 rot.
##                  814                 392                 533
apply(ps, 2, function(i)sum(p.adjust(i, "BH") < 0.05))
##             p ComBat     p Limma (+batch) p ComBat - 2000 rot.
##                  649                 271                 431
```

```
#### p-vals scatterplot

df1 <- data.frame(ps)
colnames(df1) <- colnames(ps)

F1 <- function(...){
    ggally_points(..., size = 0.7)+
        scale_y_log10()+
        scale_x_log10()+
        geom_abline(slope = 1, intercept = 0, lty = 2, lwd = 0.5, col = "red")
}
lower.pan <- list(continuous = F1, combo = "facethist", discrete = "facetbar",
                  na = "na")

my.cor <- function(...)
    ggally_statistic(
      text_fn =
        function(x,y)formatC(cor(log(x),log(y)), digits = 3, format = "f"),
      title = "Corr",
      sep = ":\n",...)

upper.pan <- list(continuous = my.cor, combo = "box_no_facet",
                  discrete = "count", na = "na")

ggpairs(df1, lower = lower.pan, upper = upper.pan, diag = NULL)
```

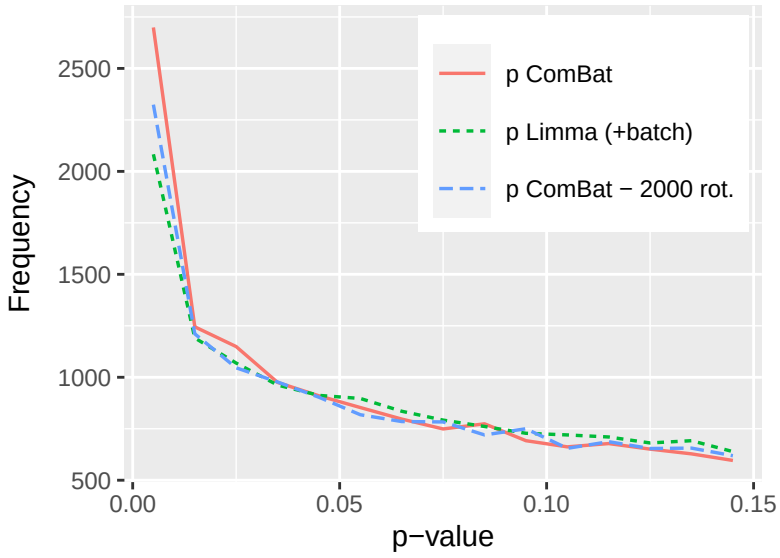



```
#### Histograms

df2 <- reshape2::melt(ps)

ggplot(df2, aes(x=value))+
    geom_histogram(colour="black", fill="lightgreen", binwidth =0.1,boundary=0)+
    facet_grid(Var2 ~ ., switch = "y")+
    geom_abline(slope = 0, intercept = nrow(ps)/10, lty = 2, col = "blue")+
    theme(axis.title.y = element_text(vjust=+3.3))
```




```
## Histograms 2

ind <- 1:15
h.com <- hist(ps.com, breaks = 100, plot = FALSE)
h.lim <- hist(ps.lim, breaks = 100, plot = FALSE)
h.rot <- hist(ps.rot, breaks = 100, plot = FALSE)

maxcount <- max(h.com$counts, h.lim$counts, h.rot$counts)

lab <- factor(rep(colnames(ps), rep(length(ind),3)), levels = colnames(ps))

df1 <- data.frame(
  mids = c(h.com$mids[ind], h.lim$mids[ind], h.rot$mids[ind]),
  counts = c(h.com$counts[ind], h.lim$counts[ind], h.rot$counts[ind]),
  lab = lab)
```

```
ggplot(df1, aes(x = mids, y = counts, colour = lab, lty = lab))+
    geom_line(lwd = 0.6) +
    xlab("p-value")+ ylab("Frequency")+
    theme(axis.title.y = element_text(vjust=+3.3)) +
    theme(legend.justification=c(1,1), legend.position=c(0.98, 0.98),
          legend.title = element_blank())+
    theme(legend.key.size = unit(1.5,"line"))
```



1 Session Info

```
sessionInfo()
## R Under development (unstable) (2020-11-14 r79432)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19041)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=German_Austria.1252  LC_CTYPE=German_Austria.1252
## [3] LC_MONETARY=German_Austria.1252 LC_NUMERIC=C
## [5] LC_TIME=German_Austria.1252
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] randRotation_1.3.1  qvalue_2.23.0       sva_3.39.0
##  [4] BiocParallel_1.25.1 genefilter_1.73.0   mgcv_1.8-33
##  [7] nlme_3.1-150        limma_3.47.0        GGally_2.0.0
## [10] ggplot2_3.3.2       BiocStyle_2.19.0
```

```
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.5           locfit_1.5-9.4       lattice_0.20-41
##  [4] snow_0.4-3           digest_0.6.27        R6_2.5.0
##  [7] plyr_1.8.6           stats4_4.1.0         RSQLite_2.2.1
## [10] evaluate_0.14        httr_1.4.2           pillar_1.4.6
## [13] Rdpack_2.1           rlang_0.4.8          annotate_1.69.0
## [16] blob_1.2.1           S4Vectors_0.29.3     Matrix_1.2-18
## [19] rmarkdown_2.5        labeling_0.4.2       splines_4.1.0
## [22] stringr_1.4.0        bit_4.0.4            munsell_0.5.0
## [25] compiler_4.1.0       xfun_0.19            pkgconfig_2.0.3
## [28] BiocGenerics_0.37.0  htmltools_0.5.0      tibble_3.0.4
## [31] bookdown_0.21        edgeR_3.33.0         IRanges_2.25.2
## [34] matrixStats_0.57.0   XML_3.99-0.5         reshape_0.8.8
## [37] crayon_1.3.4         withr_2.3.0          rbibutils_1.4
## [40] grid_4.1.0           xtable_1.8-4         gtable_0.3.0
## [43] lifecycle_0.2.0      DBI_1.1.0            magrittr_1.5
## [46] scales_1.1.1         stringi_1.5.3        farver_2.0.3
## [49] reshape2_1.4.4       xml2_1.3.2           ellipsis_0.3.1
## [52] vctrs_0.3.4          RColorBrewer_1.1-2   tools_4.1.0
## [55] bit64_4.0.5          Biobase_2.51.0       glue_1.4.2
## [58] parallel_4.1.0       survival_3.2-7       yaml_2.2.1
## [61] AnnotationDbi_1.53.0 colorspace_2.0-0     BiocManager_1.30.10
## [64] gbRd_0.4-11          memoise_1.1.0        knitr_1.30
```