Testing logarithmic fold changes at a nonzero threshold

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1 Running DE analysis with and without shrinkage of LFCs

Here we run a standard DE analysis, once with shrunken LFCs and once with unshrunken LFCs. The unshrunken LFCs are necessary in order to perform the hypothesis testing in which the alternate hypothesis is *small* LFCs. The software does not allow the combination of a prior and a test for *small* LFCs, because the prior would then support the alternate hypothesis.

```
library("DESeq2")
library("DESeq2paper")
```

```
data("bottomly_sumexp")
dds <- DESeqDataSetFromMatrix(assay(bottomly), DataFrame(colData(bottomly)),
    ~strain)
dds <- DESeq(dds)

## estimating size factors
## estimating dispersions
## gene-wise dispersion estimates
## mean-dispersion relationship
## final dispersion estimates
## fitting model and testing
## -- replacing outliers and refitting for 60 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds))
## estimating dispersions
## fitting model and testing

ddsNoPrior <- DESeq(dds, betaPrior = FALSE)

## using pre-existing size factors
## estimating dispersions
## you had estimated dispersions, replacing these
## gene-wise dispersion estimates
## mean-dispersion relationship
## final dispersion estimates
## fitting model and testing
## -- replacing outliers and refitting for 67 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds))
## estimating dispersions
## fitting model and testing

theta <- 1
res <- results(dds, lfcThreshold = theta, altHypothesis = "greaterAbs")
padj <- res$padj
```

```
padj[is.na(padj)] <- 1
resNoPrior <- results(ddsNoPrior, lfcThreshold = theta, altHypothesis = "lessAbs")
padjAB <- resNoPrior$padj
padjAB[is.na(padjAB)] <- 1
```

2 Plot

```
line <- 0.75
adj <- -0.35
cex <- 1.5

par(mfrow = c(1, 2), mar = c(4.5, 4.5, 3, 1))
ymax <- 3

plotMA(res, ylim = c(-ymax, ymax), colNonSig = rgb(0, 0, 0, 0.5), colSig = rgb(1,
    0, 0, 0.5), main = expression(H[A]:~abs(beta) > 1), colLine = NULL, ylab = expression(log[2] ~
    fold ~ change))
abline(h = c(-1, 1) * theta, col = "dodgerblue", lty = 3, lwd = 3)
legend("bottomright", "adj. p < .1", pch = 16, col = "red", bg = "white", cex = 0.8)
mtext("A", side = 3, line = line, adj = adj, cex = cex)

plotMA(resNoPrior, ylim = c(-ymax, ymax), colNonSig = rgb(0, 0, 0, 0.5), colSig = rgb(1,
    0, 0, 0.5), main = expression(H[A]:~abs(beta) < 1), colLine = NULL, ylab = expression(log[2] ~
    fold ~ change))
abline(h = c(-1, 1) * theta, col = "dodgerblue", lty = 3, lwd = 3)
legend("bottomright", "adj. p < .1", pch = 16, col = "red", bg = "white", cex = 0.8)
mtext("B", side = 3, line = line, adj = adj, cex = cex)
```

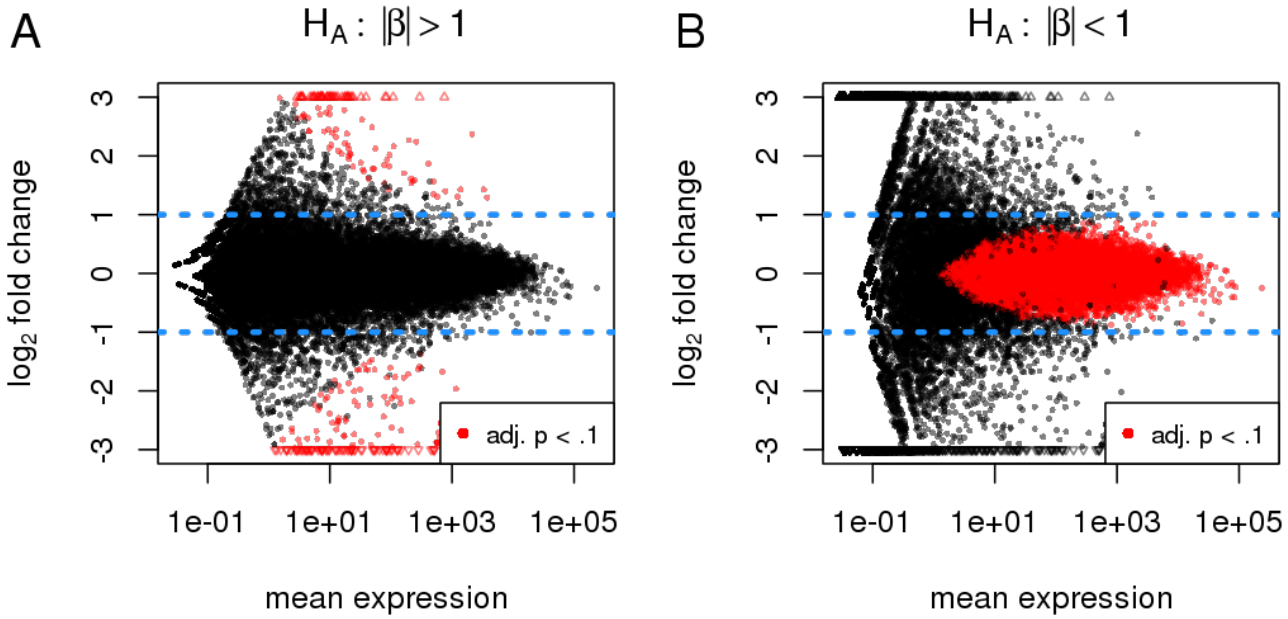

Figure 1: Testing for logarithmic fold changes above (A) and below (B) a positive threshold, using the Bottomly et al dataset. Testing for logarithmic fold changes below a threshold (B) requires that a prior on logarithmic fold changes not be used.

3 Session information

- R version 3.1.0 (2014-04-10), `x86_64-unknown-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Base packages: base, datasets, grDevices, graphics, grid, methods, parallel, stats, utils
- Other packages: BiocGenerics 0.10.0, DESeq2 1.4.0, DESeq2paper 1.3, GenomeInfoDb 1.0.0, GenomicRanges 1.16.0, IRanges 1.21.45, LSD 2.5, MASS 7.3-31, RColorBrewer 1.0-5, Rcpp 0.11.1, RcppArmadillo 0.4.200.0, colorRamps 2.3, ellipse 0.3-8, ggplot2 0.9.3.1, gridExtra 0.9.1, gtools 3.3.1, hexbin 1.27.0, knitr 1.5, schoolmath 0.4, xtable 1.7-3
- Loaded via a namespace (and not attached): AnnotationDbi 1.26.0, Biobase 2.24.0, DBI 0.2-7, RSQLite 0.11.4, XML 3.98-1.1, XVector 0.4.0, annotate 1.42.0, codetools 0.2-8, colorspace 1.2-4, dichromat 2.0-0, digest 0.6.4, evaluate 0.5.5, formatR 0.10, genefilter 1.46.0, geneplotter 1.42.0, gtable 0.1.2, highr 0.3, labeling 0.2, lattice 0.20-29, locfit 1.5-9.1, munsell 0.4.2, plyr 1.8.1, proto 0.3-10, reshape2 1.4, scales 0.2.3, splines 3.1.0, stats4 3.1.0, stringr 0.6.2, survival 2.37-7, tools 3.1.0