

Additional plots for: Independent filtering increases power for detecting differentially expressed genes, Bourgon et al., PNAS (2010)

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1 Introduction

This vignette illustrates use of some functions in the *genefilter* package that provide useful diagnostics for independent filtering [1]:

- `kappa_p` and `kappa_t`
- `filtered_p` and `filtered_R`
- `filter_volcano`
- `rejection_plot`

2 Data preparation

Load the ALL data set and the *genefilter* package:

```
library("genefilter")
library("ALL")
data("ALL")
```

Reduce to just two conditions, then take a small subset of arrays from these, with 3 arrays per condition:

```
bcell <- grep("^B", as.character(ALL$BT))
moltyp <- which(as.character(ALL$mol.biol) %in%
               c("NEG", "BCR/ABL"))
ALL_bcrneg <- ALL[, intersect(bcell, moltyp)]
ALL_bcrneg$mol.biol <- factor(ALL_bcrneg$mol.biol)
```

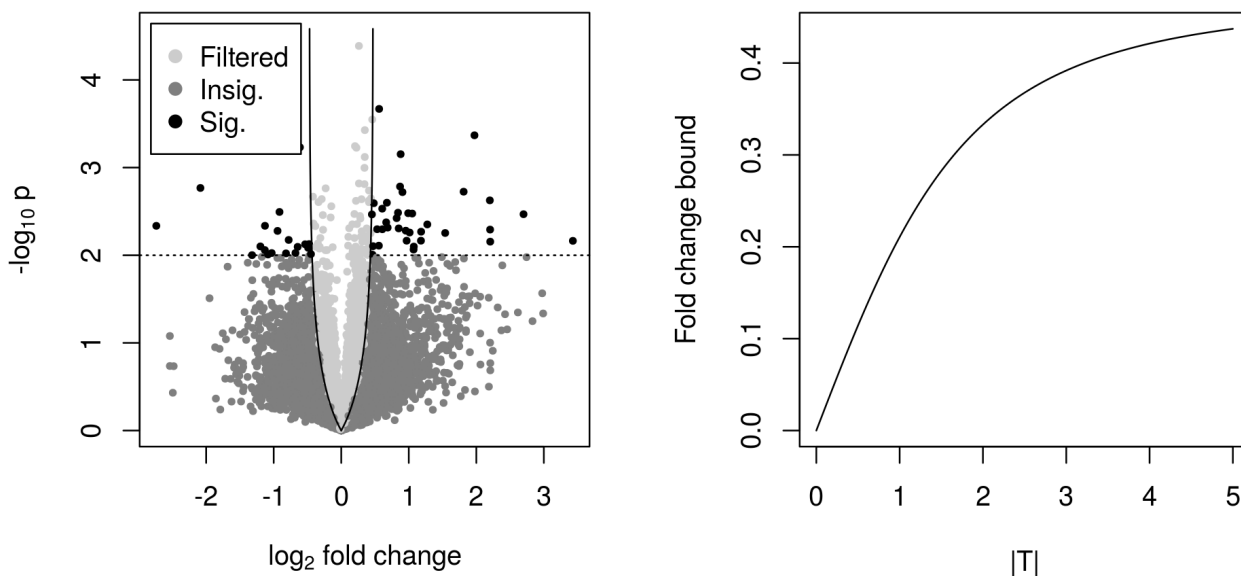


Figure 1: Left panel: plot produced by the `filter_volcano` function. Right panel: graph of the `kappa_t` function.

```
n1 <- n2 <- 3
set.seed(1969)
use <- unlist(tapply(1:ncol(ALL_bcrneg),
                    ALL_bcrneg$mol.biol, sample, n1))
subsample <- ALL_bcrneg[,use]
```

We now use functions from *genefilter* to compute overall standard deviation filter statistics as well as standard two-sample t and related statistics.

```
S <- rowSds( exprs( subsample ) )
temp <- rowttests( subsample, subsample$mol.biol )
d <- temp$dm
p <- temp$p.value
t <- temp$statistic
```

3 Filtering volcano plot

Filtering on overall standard deviation and then using a standard t -statistic induces a lower bound of fold change, albeit one which varies somewhat with the significance of the t -statistic. The `filter_volcano` function allows you to visualize this effect.

The output is shown in the left panel of Fig. 1.

The `kappa_p` and `kappa_t` functions, used to make the volcano plot, compute the fold change bound multiplier as a function of either a t -test p -value or the t -statistic itself. The actual induced bound on the fold change is κ times the filter's cutoff on the overall standard deviation. Note that fold change bounds for values of $|T|$ which are close to 0 are not of practical interest because we will not reject the null hypothesis with test statistics in this range.

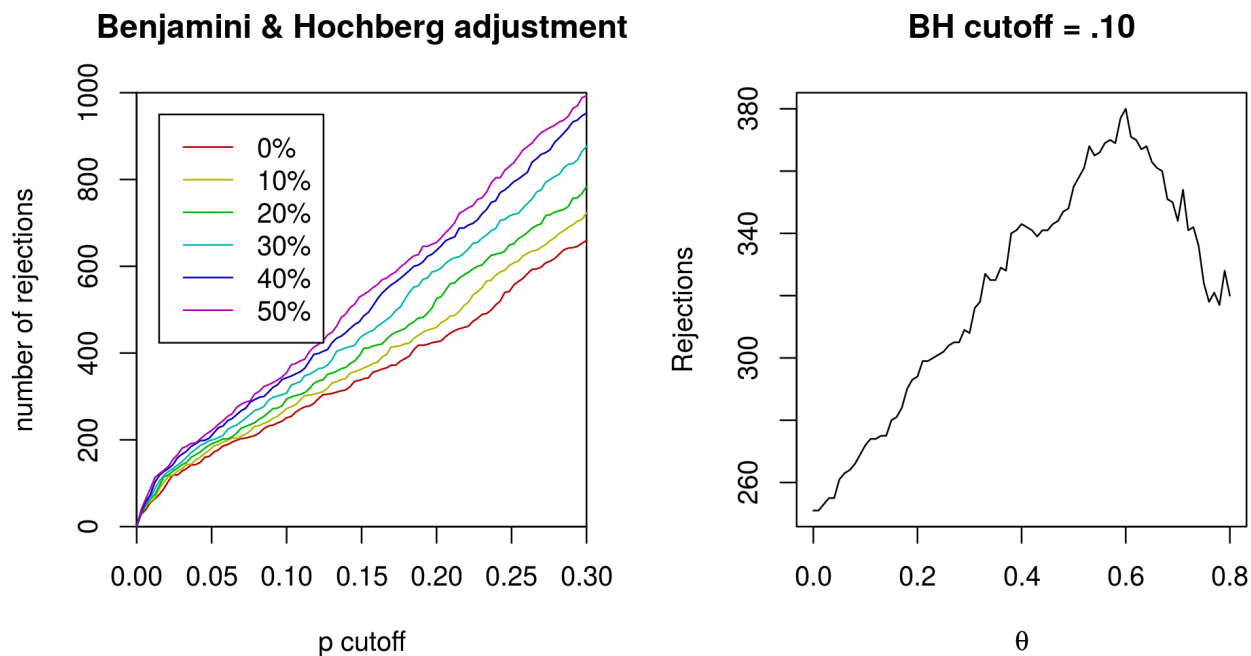


Figure 2: Left panel: plot produced by the `rejection_plot` function. Right panel: graph of θ .

The plot is shown in the right panel of Fig. 1.

4 Rejection count plots

4.1 Across p -value cutoffs

The `filtered_p` function permits easy simultaneous calculation of unadjusted or adjusted p -values over a range of filtering thresholds (θ). Here, we return to the full “BCR/ABL” versus “NEG” data set, and compute adjusted p -values using the method of Benjamini and Hochberg, for a range of different filter stringencies.

```
table(ALL_bcrneg$mol.biol)

##
## BCR/ABL    NEG
##      37     42

S2 <- rowVars(exprs(ALL_bcrneg))
p2 <- rowttests(ALL_bcrneg, "mol.biol")$p.value
theta <- seq(0, .5, .1)
p_bh <- filtered_p(S2, p2, theta, method="BH")

head(p_bh)
```

	0%	10%	20%	30%	40%	50%
## [1,]	0.919	0.894	0.862	0.828	NA	NA
## [2,]	0.959	0.946	0.930	0.906	0.887	0.871
## [3,]	0.702	NA	NA	NA	NA	NA

```
## [4,] 0.981 0.975 0.968 0.957 NA NA
## [5,] 0.951 0.935 0.912 0.884 NA NA
## [6,] 0.634 0.590 0.544 0.495 0.450 0.410
```

The `rejection_plot` function takes sets of p -values corresponding to different filtering choices — in the columns of a matrix or in a list — and shows how rejection count (R) relates to the choice of cutoff for the p -values. For these data, over a reasonable range of FDR cutoffs, increased filtering corresponds to increased rejections.

```
rejection_plot(p_bh, at="sample",
               xlim=c(0,.3), ylim=c(0,1000),
               main="Benjamini & Hochberg adjustment")
```

The plot is shown in the left panel of Fig. 2.

4.2 Across filtering fractions

If we select a fixed cutoff for the adjusted p -values, we can also look more closely at the relationship between the fraction of null hypotheses filtered and the total number of discoveries. The `filtered_R` function wraps `filtered_p` and just returns rejection counts. It requires a p -value cutoff.

```
theta <- seq(0, .80, .01)
R_BH <- filtered_R(alpha=.10, S2, p2, theta, method="BH")
```

```
head(R_BH)
## 0% 1% 2% 3% 4% 5%
## 251 251 253 255 255 261
```

Because overfiltering (or use of a filter which is inappropriate for the application domain) discards both false and true null hypotheses, very large values of θ reduce power in this example:

```
plot(theta, R_BH, type="l",
     xlab=expression(theta), ylab="Rejections",
     main="BH cutoff = .10"
)
```

The plot is shown in the right panel of Fig. 2.

Session information

- R version 3.2.2 (2015-08-14), x86_64-pc-linux-gnu
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: ALL 1.11.0, Biobase 2.30.0, BiocGenerics 0.16.0, DESeq 1.22.0, RColorBrewer 1.1-2, genefilter 1.52.0, knitr 1.11, lattice 0.20-33, locfit 1.5-9.1, pasilla 0.9.1
- Loaded via a namespace (and not attached): AnnotationDbi 1.32.0, BiocStyle 1.8.0, DBI 0.3.1, IRanges 2.4.0, RSQLite 1.0.0, S4Vectors 0.8.0, XML 3.98-1.3, annotate 1.48.0, codetools 0.2-14, digest 0.6.8, evaluate 0.8, formatR 1.2.1, geneplotter 1.48.0, grid 3.2.2, highr 0.5.1, magrittr 1.5, splines 3.2.2, stats4 3.2.2, stringi 0.5-5, stringr 1.0.0, survival 2.38-3, tools 3.2.2, xtable 1.7-4

References

- [1] Richard Bourgon, Robert Gentleman and Wolfgang Huber. Independent filtering increases power for detecting differentially expressed genes.