

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

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*genefilter* version 1.50.0 (Last revision 2014-10-15 )

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## 1 Introduction

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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

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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)
```

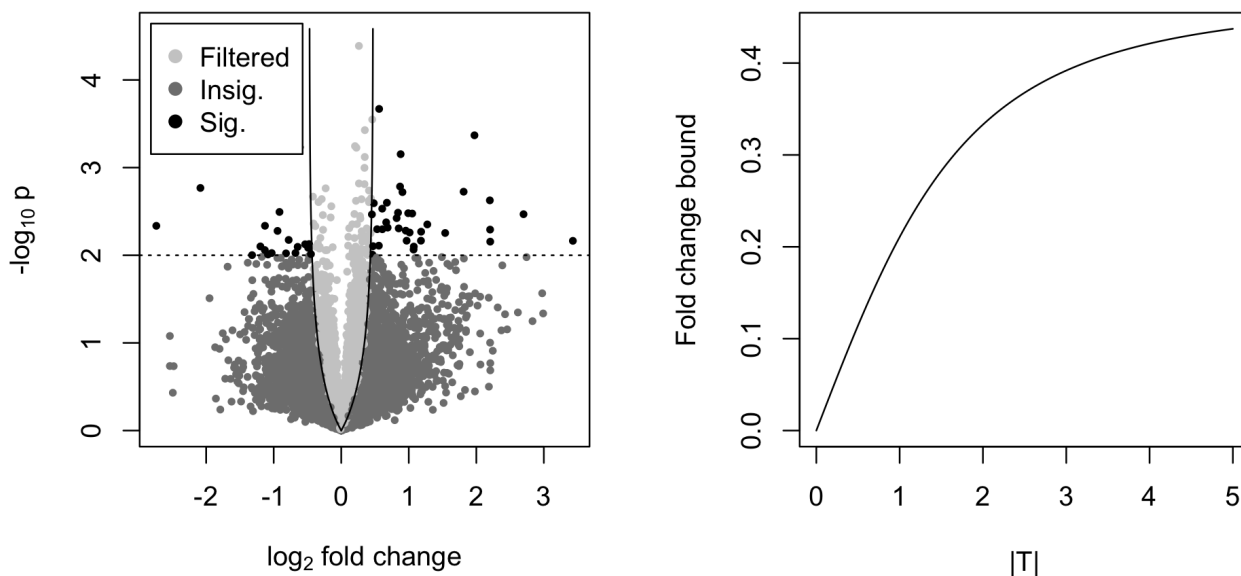


Figure 1: Left panel: plot produced by the `filter_volcano` function. Right panel: graph of the `kappa_t` function.

```
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  $\kappa$  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.

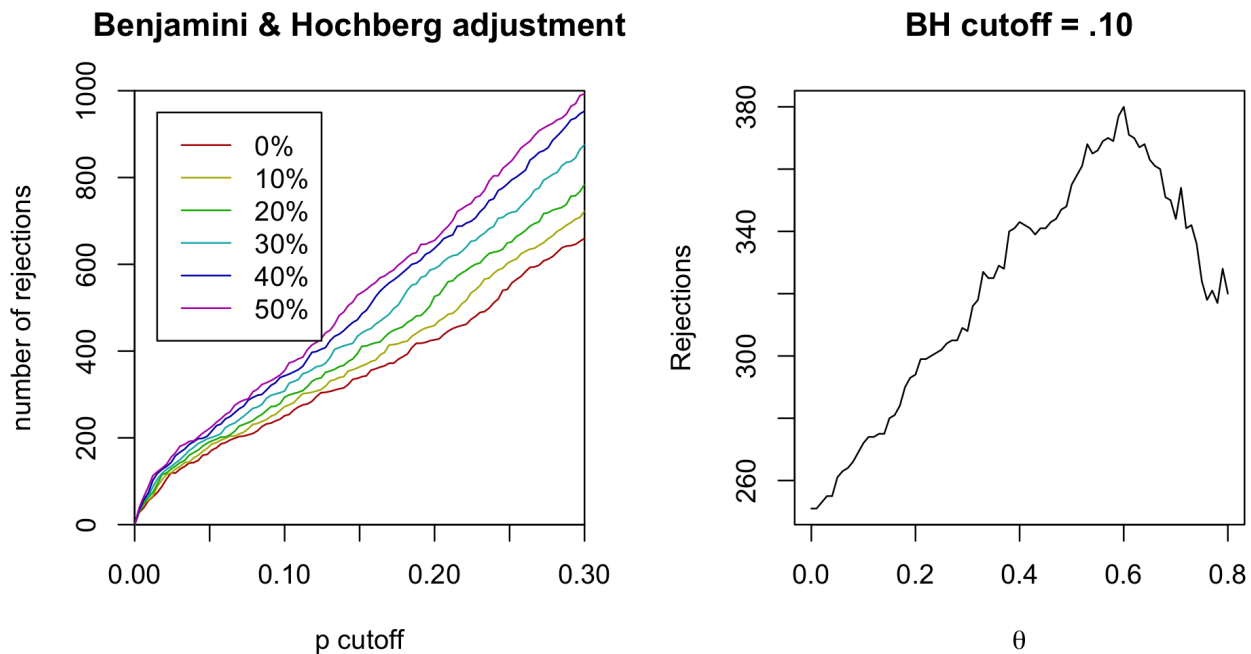


Figure 2: Left panel: plot produced by the `rejection_plot` function. Right panel: graph of  $\theta$ .

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 ( $\theta$ ). 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  $\theta$  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 3.2.0 RC (2015-04-08 r68161), x86\_64-apple-darwin13.4.0
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: ALL 1.9.1, Biobase 2.28.0, BiocGenerics 0.14.0, DESeq 1.20.0, RColorBrewer 1.1-2, genefilter 1.50.0, knitr 1.9, lattice 0.20-31, locfit 1.5-9.1, pasilla 0.7.1
- Loaded via a namespace (and not attached): AnnotationDbi 1.30.0, BiocStyle 1.6.0, DBI 0.3.1, GenomInfoDb 1.4.0, IRanges 2.2.0, RSQLite 1.0.0, S4Vectors 0.6.0, XML 3.98-1.1, annotate 1.46.0, codetools 0.2-11, digest 0.6.8, evaluate 0.6, formatR 1.1, geneplotter 1.46.0, grid 3.2.0, highr 0.4.1, splines 3.2.0, stats4 3.2.0, stringr 0.6.2, survival 2.38-1, tools 3.2.0, xtable 1.7-4

## References

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