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

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**genefilter version 1.54.2 (Last revision 2016-03-22 )**

## Contents

## 1 Introduction

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

- `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)
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 devation filter statistics as well as standard two-sample $t$ and releated 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. **??**.

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.
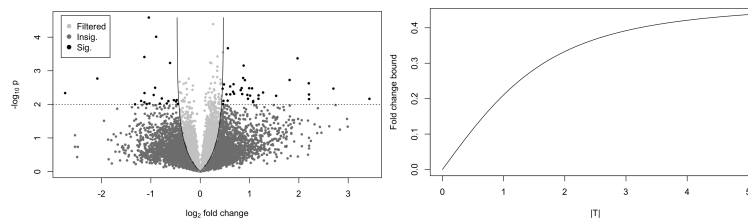


**Figure 1: Left panel: plot produced by the `filter_volcano` function.** Right panel: graph of the `kappa_t` function.

The plot is shown in the right panel of Fig. **??**.

# 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.
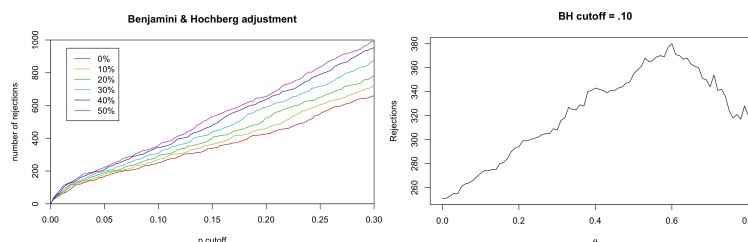


**Figure 2:** **Left panel: plot produced by the `rejection_plot` function.** Right panel: graph of `theta`.

```
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. **??**.

## 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. **??**.

# Session information

- R version 3.3.0 (2016-05-03), `x86_64-apple-darwin13.4.0`
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: ALL 1.14.0, Biobase 2.32.0, BiocGenerics 0.18.0, DESeq 1.24.0, RColorBrewer 1.1-2, genefilter 1.54.2, knitr 1.13, lattice 0.20-33, locfit 1.5-9.1, pasilla 0.12.0
- Loaded via a namespace (and not attached): AnnotationDbi 1.34.2, BiocStyle 2.0.2, DBI 0.4-1, IRanges 2.6.0, Matrix 1.2-6, RSQLite 1.0.0,

S4Vectors 0.10.0, XML 3.98-1.4, annotate 1.50.0, codetools 0.2-14, digest 0.6.9, evaluate 0.9, formatR 1.4, geneplotter 1.50.0, grid 3.3.0, highr 0.6, magrittr 1.5, splines 3.3.0, stats4 3.3.0, stringi 1.0-1, stringr 1.0.0, survival 2.39-4, tools 3.3.0, xtable 1.8-2

# References

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