

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

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*genefilter* version 1.70.0 (Last revision )

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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

---

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 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
```

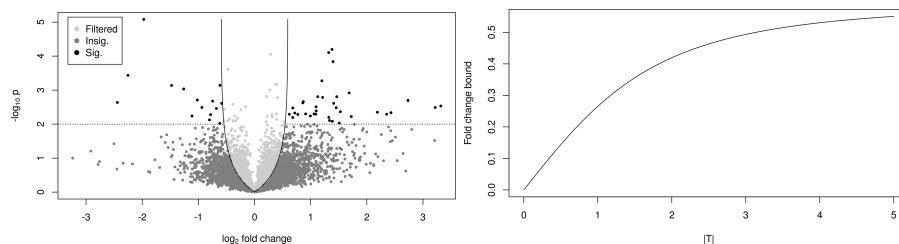
### 3 Filtering volcano plot

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.

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



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

## 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
```

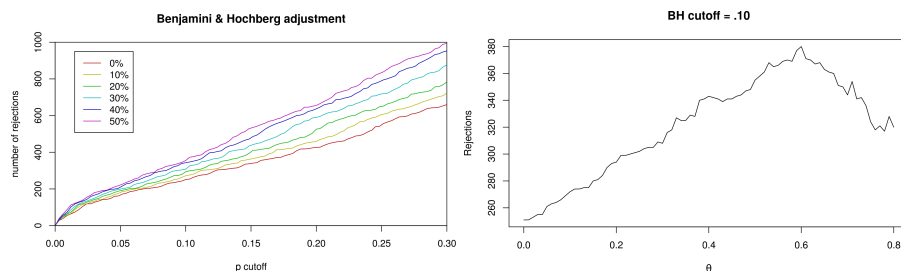
```
S2 <- rowVars(exprs(ALL_bcrneg))
p2 <- rowttests(ALL_bcrneg, "mol.biol")$p.value
theta <- seq(0, .5, .1)
p_bh <- filtered_p(S2, p2, theta, method="BH")
```

```
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")
```



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

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 4.0.0 (2020-04-24), x86\_64-pc-linux-gnu
- Running under: Ubuntu 18.04.4 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.11-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.11-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: ALL 1.29.0, Biobase 2.48.0, BiocGenerics 0.34.0, DESeq 1.40.0, RColorBrewer 1.1-2, genefilter 1.70.0, knitr 1.28, lattice 0.20-41, locfit 1.5-9.4, pasilla 1.15.0
- Loaded via a namespace (and not attached): AnnotationDbi 1.50.0, BiocManager 1.30.10, BiocStyle 2.16.0, DBI 1.1.0, IRanges 2.22.0, Matrix 1.2-18, RCurl 1.98-1.2, RSQLite 2.2.0, Rcpp 1.0.4.6, S4Vectors 0.26.0, XML 3.99-0.3, annotate 1.66.0, bit 1.1-15.2, bit64 0.9-7, bitops 1.0-6, blob 1.2.1, codetools 0.2-16, compiler 4.0.0, digest 0.6.25, evaluate 0.14, geneplotter 1.66.0, grid 4.0.0, highr 0.8,

htmltools 0.4.0, magick 2.3, magrittr 1.5, memoise 1.1.0, rlang 0.4.5, rmarkdown 2.1,  
splines 4.0.0, stats4 4.0.0, stringi 1.4.6, stringr 1.4.0, survival 3.1-12, tools 4.0.0,  
vctrs 0.2.4, xfun 0.13, xtable 1.8-4, yaml 2.2.1

## References

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