

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

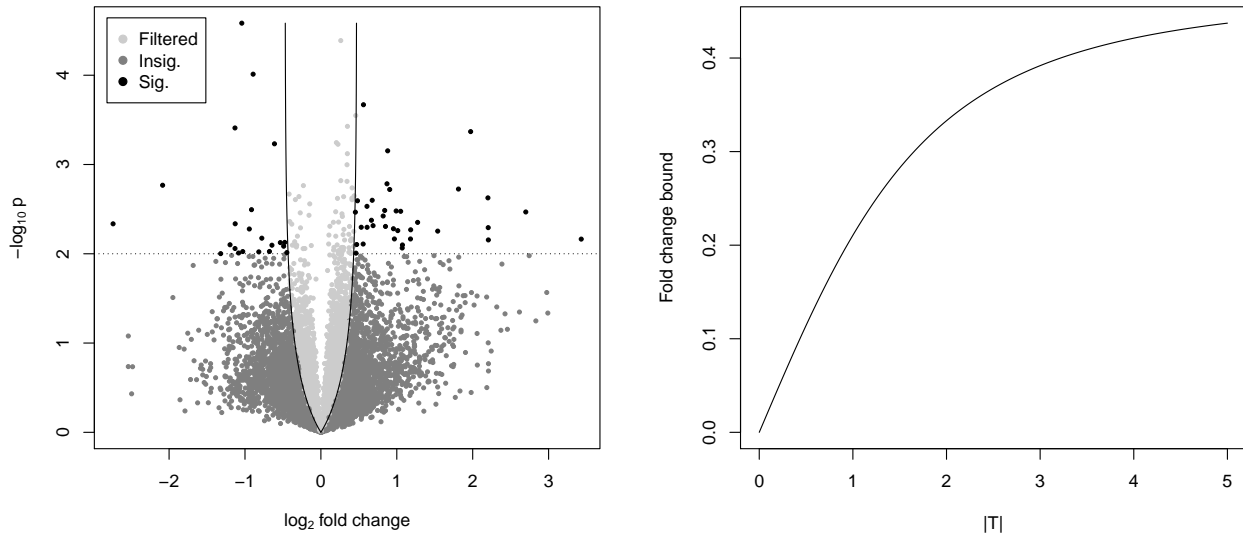


Figure 1: Left panel: plot produced by the `filter_volcano` function. Right panel: graph of the `kappa_t` function.

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.

```
> S_cutoff <- quantile(S, .50)
> filter_volcano(d, p, S, n1, n2, alpha=.01, S_cutoff)
```

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.

```
> t <- seq(0, 5, length=100)
> plot(t, kappa_t(t, n1, n2) * S_cutoff,
+       xlab="|T|", ylab="Fold change bound", type="l")
```

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.

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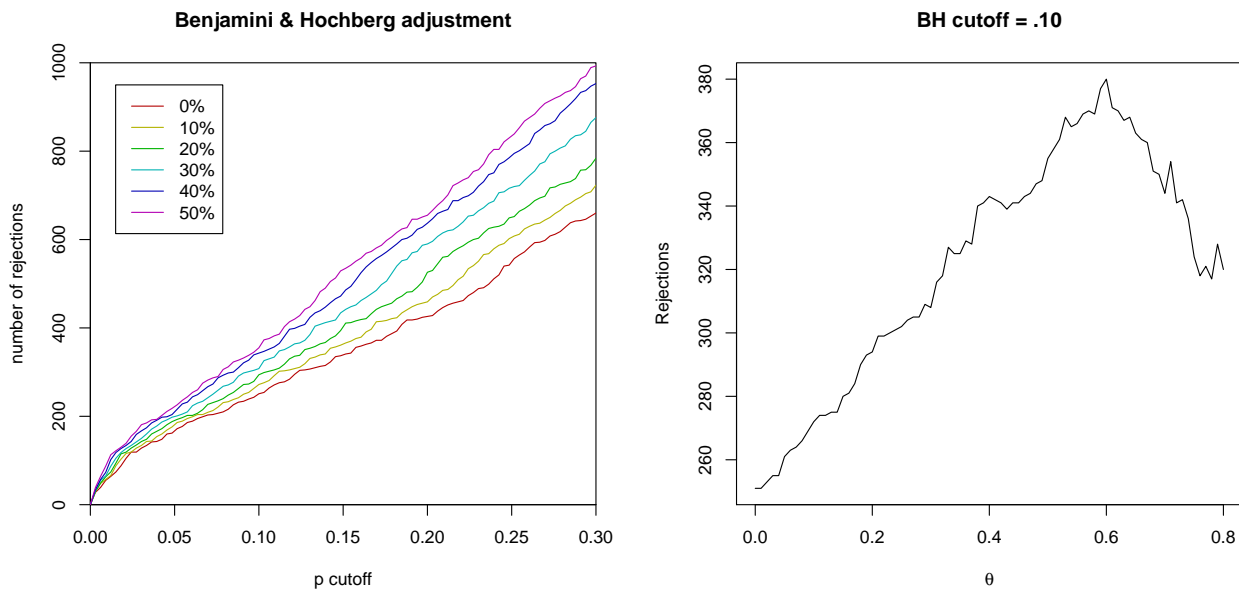


Figure 2: Left panel: plot produced by the `rejection_plot` function. Right panel: graph of θ .

```
> 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")
```

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```
> 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 3.1.0 (2014-04-10), x86_64-apple-darwin13.1.0
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, utils
- Other packages: ALL 1.6.0, Biobase 2.24.0, BiocGenerics 0.10.0, DESeq 1.16.0, RColorBrewer 1.0-5, class 7.3-10, genefilter 1.46.1, lattice 0.20-29, locfit 1.5-9.1, pasilla 0.4.0
- Loaded via a namespace (and not attached): AnnotationDbi 1.26.0, DBI 0.2-7, GenomeInfoDb 1.0.2, IRanges 1.22.6, RSQLite 0.11.4, XML 3.98-1.1, annotate 1.42.0, geneplotter 1.42.0, grid 3.1.0, splines 3.1.0, stats4 3.1.0, survival 2.37-7, tools 3.1.0, xtable 1.7-3

References

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