Supporting Information 1: Impact of filtering of protein/peptide lists on FDR estimates.

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Introduction

Some may assume that shortening the list of proteins would artificially increase the false discovery rate (FDR). However, the FDR, estimated using the Benjamini-Hochberg correction, depends primarily on the p-value distribution relative to the null hypothesis (H_0, a uniform distribution), not the absolute number of proteins tested. While methods controlling the Family-Wise Error Rate (FWER) are sensitive to the total number of tests, the FDR estimation is unaffected as long as the p-value distribution remains unchanged.

The computer Experiment

Example 1, filtering without changing the distribution of p-values

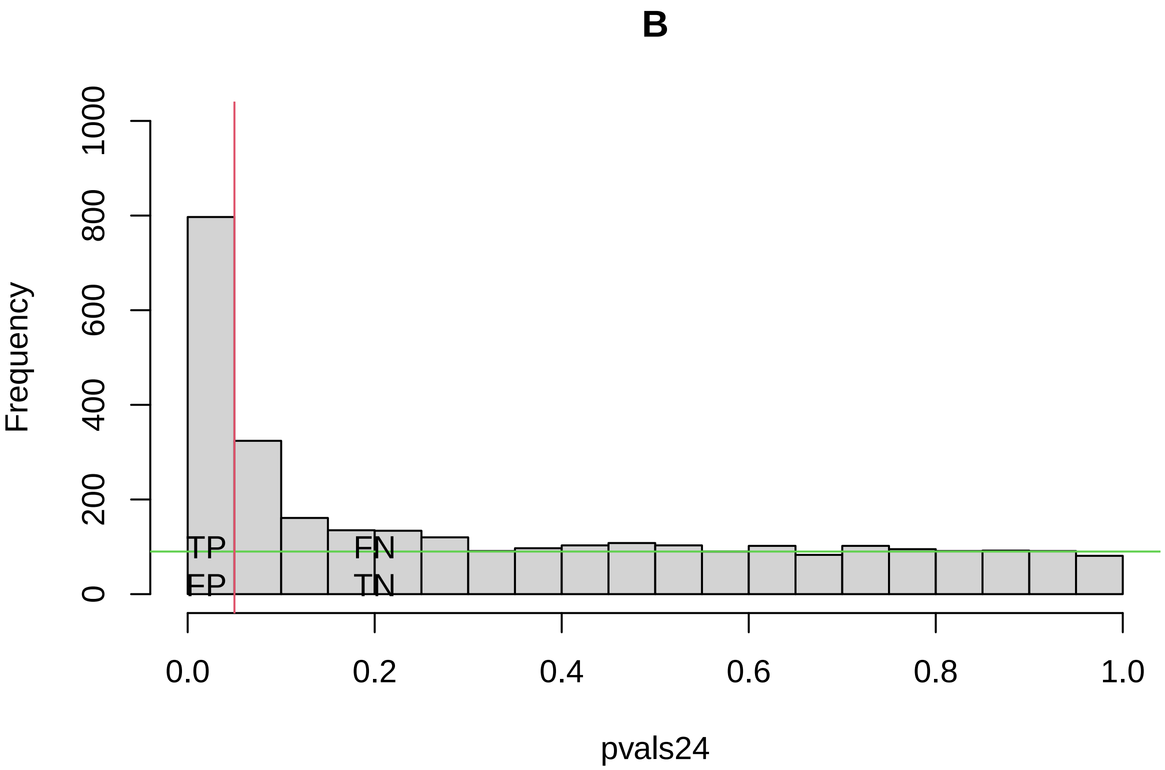
We start by simulating a list of $m = 3000$ p-values. 90% datapoints comes from H0, 10% from H1 (fold change of 2). The group sizes are 4. We store these p-values in the array $pvals24$.

```
m <- 3000
simulate.p.values <- function(
    i, delta = 2, fraction = 0.1, ss = 4){
  control <- rnorm(ss,0,1)
  treatment <- rnorm(ss,0,1)
  if (runif(1) < fraction)
    treatment <- treatment + delta
  return(t.test(treatment,control)$p.value)
}
pvals24 <- sapply(1:m, simulate.p.values,
                  delta = 2, fraction = 0.4 )
```

We plot the distribution of the p-values and see that we can estimate FP TP FN and TN from the histogram, which allows us to compute the false discovery proportion (FDP).

$$FDP = \frac{FP}{FP + TP}$$

```
hist(pvals24, breaks = 20, ylim = c(0,m/3), main = "B")
abline(h = (m - m * 0.4)/20, col = 3)
abline(v = 0.05, col = 2)
text(x = c(0.02, 0.02, 0.2, 0.2),y = c(20,100,20,100), labels = c("FP","TP","TN","FN"))
```



We can also compute the FDR using the Benjamini-Hochberg method using the function *p.adjust*.

```
xx <- data.frame(p.vals = pvals24, FDR = p.adjust(pvals24, method = "BH"))
```

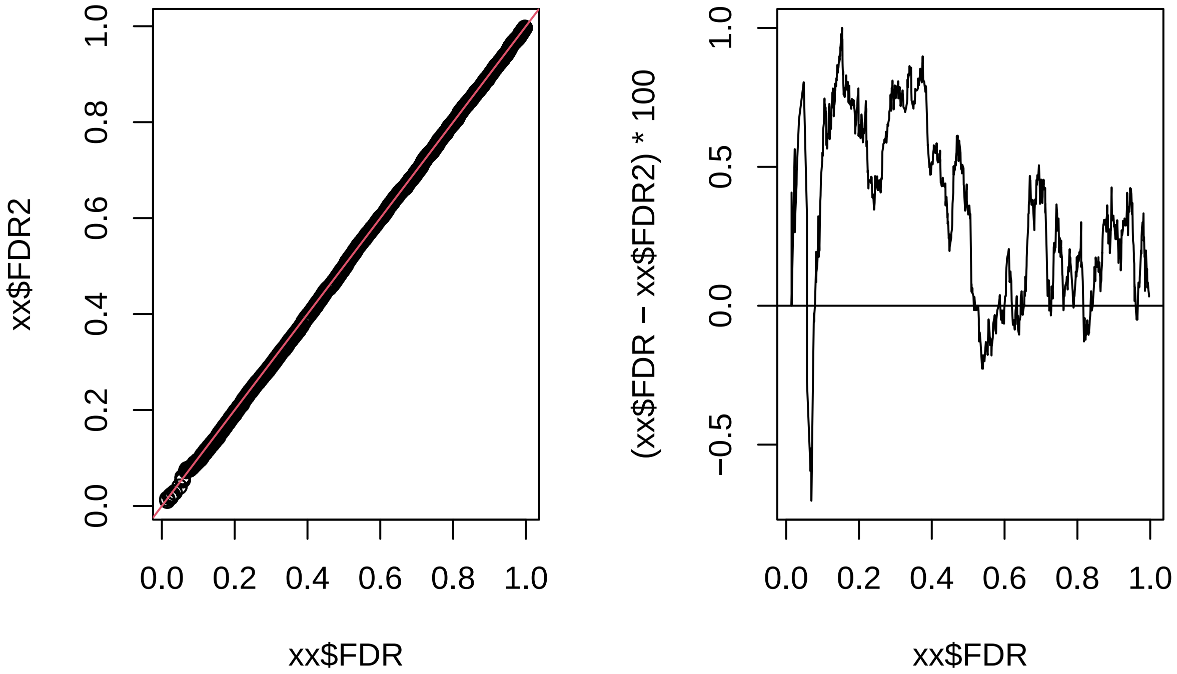
In proteomics experiments, only a single peptide identifies approximately 1/3 of proteins. If we remove these proteins from the list, and keep only those quantified by two or more peptides, do we need to recompute the FDR?

We filter the simulated p-values, and keep only 2/3 of them. We randomly choose which rows of data to use. We then compute the FDR from the remaining $p - values$.

```
xx <- xx[sample(1:m,floor(m*2/3)),]
xx$FDR2 <- p.adjust(xx$p.vals, method = "BH")
```

We compare the FDR estimates computed for all 3000 proteins and the 2000 proteins left after filtering. We can see that the differences in the p-value estimates are less than 1.

```
par(mfrow = c(1,2))
xx <- xx[order(xx$p.vals),]
plot(xx$FDR,xx$FDR2)
abline(c(0,1), col = 2)
plot(xx$FDR, (xx$FDR - xx$FDR2)*100, pch = ".", type = "l")
abline(h = 0)
```



We examine how this affects the list of selected proteins if we filter for FDR at 5%, 10%, or 25%.

```
ll <- data.frame(FDR = c("5" = sum(xx$FDR < 0.05),
"10" = sum(xx$FDR < 0.1), "25" = sum(xx$FDR < 0.25)),

FDR2 = c("5" = sum(xx$FDR2 < 0.05),"10" = sum(xx$FDR2 < 0.1),  "25" = sum(xx$FDR2 < 0.25)))
knitr::kable(ll)
```

	FDR	FDR2
5	16	16
10	235	265
25	723	735

We see that we end up with similar lists of proteins, and it does not matter if we used the FDR computed before or after removing 1/3 of observations.

Example 2, filtering and changing the distribution of p-values.

We now change the distribution of the p-values by filtering the list of proteins and then recomputing the FDR.

We augment the original list of 3000 p-values with 10 p.values equal to 1 and compute the FDR.

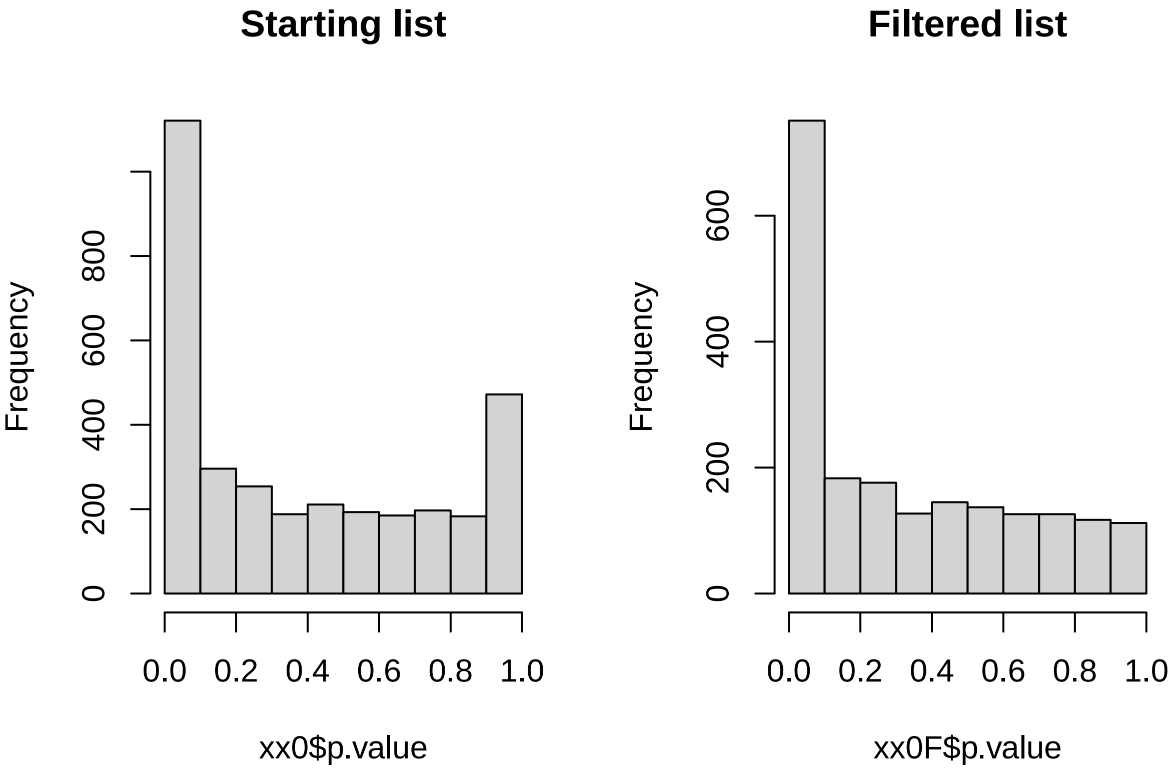
```
addP <- ceiling(m*0.1)
pvals240 <- c(pvals24, rep(1,addP))
xx0 <- data.frame(p.value = pvals240, FDR = p.adjust(pvals240, method = "BH"))
```

Next, we remove those p-values equal 1, and in addition 33% of the other p-values, leaving 2000 p-values.

```
xx0F <- xx0[-(3001:(3000 + addP)),]
xx0F <- xx0F[sample(1:m,floor(m*2/3)),]
```

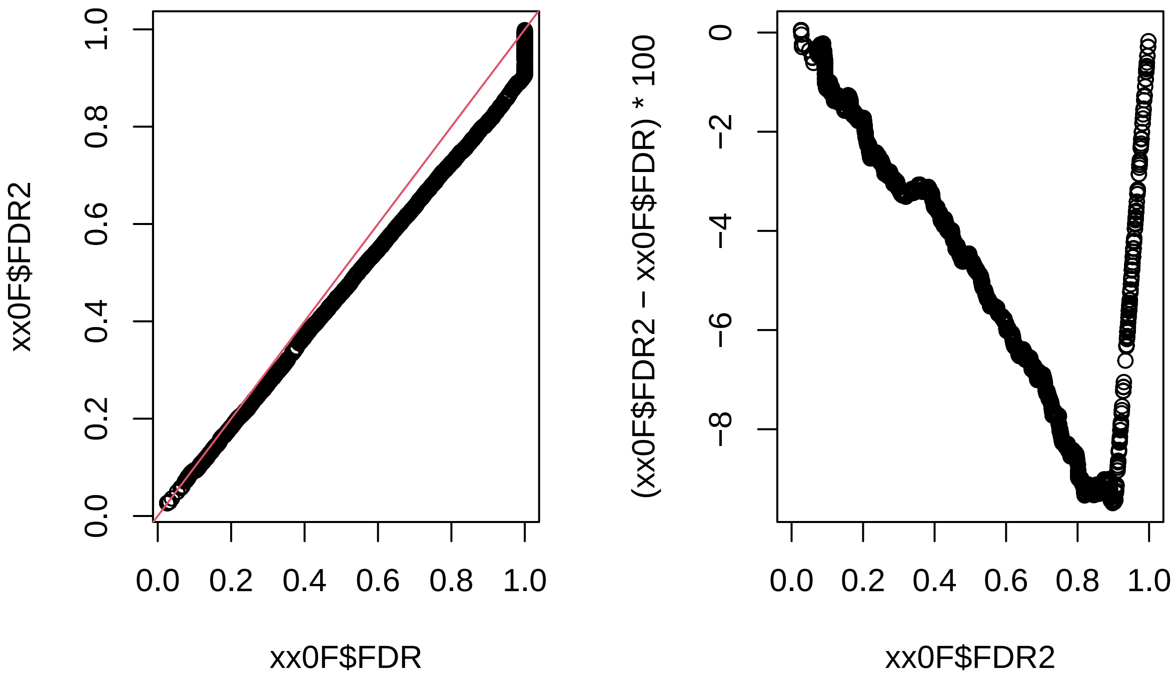
Clearly, the p-value distributions do differ before and after filtering.

```
par(mfrow = c(1,2))
hist(xx0$p.value, main = "Starting list")
hist(xx0F$p.value, main = "Filtered list")
```



Now we do recompute the FDR from the truncated list of p-values, and compare the FDR estimates computed before with those computed after filtering the lists.

```
xx0F$FDR2 <- p.adjust(xx0F$p.value, method = "BH")
par(mfrow = c(1,2))
plot(xx0F$FDR, xx0F$FDR2)
abline(0,1,col = 2)
plot(xx0F$FDR2,(xx0F$FDR2 - xx0F$FDR)*100, xlim = c(0,1))
```



The difference between the FDR computed before and after filtering is substantial. Again, we examine, how this affects the list of selected proteins if we filter for FDR at 5%, 10% or 25%.

```
ll <- data.frame(FDR = c("5" = sum(xx0F$FDR < 0.05),
"10" = sum(xx0F$FDR < 0.1), "25" = sum(xx0F$FDR < 0.25)),

FDR2 = c("5" = sum(xx0F$FDR2 < 0.05),"10" = sum(xx0F$FDR2 < 0.1),  "25" = sum(xx0F$FDR2 < 0.25)))
knitr::kable(ll)
```

	FDR	FDR2
5	11	13
10	137	223
25	671	713

The lists of proteins/peptides filtered by FDR at 5%, 10%, and 25 have different lengths and become relatively smaller when FDR thresholds are larger.