1 Introduction

In the context of genome-wide studies for which a large number of statistical tests are simultaneously performed, the False Discovery Rate (FDR) which is defined as the expected proportion of false discoveries \( \frac{\pi_0 \Pr(P \in G | H = 0)}{\Pr(P \in G)} \) is one of the most used criterion for taking into account the multiple testing problem.

In the framework of estimating procedures based on the marginal distribution of the p-values without assumption on the conditional distribution related to the alternative hypothesis, estimators of the FDR rely on the formula introduced by Storey [4]:

\[
FDR(\Gamma) = \frac{\pi_0 \Pr(P \in G | H = 0)}{\Pr(P \in G)}
\]

where \( H \) is the variable such that \( H = 0 \) if the null hypothesis \( H_0 \) is true, \( H = 1 \) if the alternative hypothesis \( H_1 \) is true, \( \pi_0 = \Pr(H = 0) \) is the probability of not being modified and \( P \) is the random variable corresponding to the p-values.

This document provides a tutorial for using the LBE package that contains functions for estimating the proportion of true null hypotheses \( \pi_0 \) and the FDR (or the q-values that are defined for each p-value by q-value\( (p_i) = FDR([0, p_i]) \). We describe here the implemented functions and illustrate their use with the real dataset from Golub et al. [3]. In the last section, the LBE function is compared with the qvalue function from the qvalue package.

2 The leukemia data set from Golub et al. (1999)

The aim of the study of Golub et al. [3] was to identify differentially expressed genes between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). The samples were assayed using Affymetrix Hgu6800 chips and the data on the expression of 7129 genes are available in the Bioconductor package golubEsets.

The dataset `golub.pval` provided with the LBE package contains the p-values obtained from a two-sample t-test analysis. The variance-stabilizing method included in the vsn package was applied for normalizing the data.

\[
\text{library(LBE)}
\]
\[
\text{data(golub.pval)}
\]
3 Implemented functions

3.1 LBE

Under the null hypothesis, the p-values are supposed to be uniformly distributed on $[0,1]$ so that

$$\Pr(P \in [0,\gamma]|H = 0) = \gamma.$$  

The FDR estimation is then obtained from the relation (1) by the separate estimation of $\Pr(P \in [0,\gamma])$ and $\pi_0$, the proportion of true null hypotheses. While $\Pr(P \in [0,\gamma])$ can easily be estimated by the empirical cumulative distribution, if no distributional assumption is made for the marginal distribution of the p-values, only an upper bound estimate can be obtained for $\pi_0$.

Let $m$ be the total number of p-values. From a classical two-components mixture model for the distribution of the p-values, we have introduced [2] a conservatively biased estimator of $\pi_0$:

$$\hat{\pi}_0 = \frac{2}{m} \sum_{i=1}^{m} P_i \quad (2)$$

From this estimator of $\pi_0$, we have demonstrated that under suitable conditions for a function $\varphi$, transformed p-values lead to a less biased estimator of $\pi_0$ (see the theorem 1 in [2]). In this context, we have considered the functions $\varphi_a(P) = -\ln(1-x)^a$, $a \in [1,+\infty]$, and we have demonstrated that these functions lead to a family of estimators for which the bias for $\pi_0$ is decreasing with $a$. The obtained results can easily be extended to real values of $a$ leading to the following family of estimators:

$$\hat{\pi}_{0(a)} = \frac{1}{m} \sum_{i=1}^{m} \frac{[-\ln(1-p_i)]^a}{\Gamma(a+1)}, \quad a \in [1,+\infty]. \quad (3)$$

For this family of estimators, an upper bound of the asymptotic variance can be obtained for independent p-values: $\frac{1}{m} \times \left( \frac{\Gamma(2a+1)}{\Gamma(a+1)^2} - 1 \right)$ leading to a confidence interval for $\pi_0$. As there is a balance between bias (decreasing as $a$ increase) and variance (increasing as $a$ increase), for a specified number $m$ of tested hypotheses, we have proposed to choose $a$ as the greatest value such that the upper bound of the standard deviation is less than a threshold $l$ for the variance’s upper bound. Other rules may obviously be considered and the LBE function allows to set $a$ independently from the variance upper bound.

The LBE function only requires a vector of p-values as input. We first create the object `LBE.res` by applying the LBE function with default arguments. A plot of the q-values versus the p-values is displayed together with the histogram of the p-values and informative numerical values in the legend such as the FDR and the number of rejected null hypotheses. Among the saved results `LBE.res`, we display the estimate of $\pi_0$, its confidence interval and the (default) level for the confidence interval. Then, we apply the LBE function once again by changing the level for the confidence interval and we display the new confidence interval for $\pi_0$. The argument plot.type is set to “none” so that the plot is not displayed.

```r
> LBE.res <- LBE(golub.pval)
```
> # LBE.res <- LBE(golub.pval)
> names(LBE.res)

[1] "call"    "FDR"     "pi0"     "pi0.ci"
[5] "ci.level" "a"       "l"       "qvalues"
[9] "pvalues"  "significant" "n.significant"

> LBE.res$pi0; LBE.res$pi0.ci; LBE.res$ci.level

[1] 0.6022541

[1] 0.0000000 0.6844972

[1] 0.95

> LBE.res2 <- LBE(golub.pval, ci.level=0.8, plot.type="none")
> LBE.res2$pi0.ci; LBE.res2$ci.level
If the argument `qvalues` is set to `FALSE`, only $\pi_0$ is estimated. Otherwise (default value), the q-values (which are defined for each gene by $q(p_i) = FDR([0,p_i]$) are estimated and the LBE function returns either the number of significant genes when controlling the FDR at a specific level (the default value for the argument `FDR.level` is 0.05), either the estimated FDR for a specific number of significant genes.

First, we apply the LBE function by changing the level at which control the FDR, then we estimate the FDR when 300 genes are declared significant. It is worth noting that the estimated q-values remain unchanged.

```r
> LBE.res3 <- LBE(golub.pval, FDR.level=0.1,plot.type="none")
> LBE.res3$qvalues[1:10]
[1] 0.1725651 0.5072883 0.4221644 0.1578850 0.4381401 0.5353073 0.2620085
[8] 0.1581277 0.2720757 0.4948859
> LBE.res3$n.significant

> LBE.res4 <- LBE(golub.pval,FDR.level=NA,n.significant=300)
```
> `LBE.res4$qvalues[1:10]`

[1] 0.1725651 0.5072883 0.4221644 0.1578850 0.4381401 0.5353073 0.2620085
[8] 0.1581277 0.2720757 0.4948859

Using the proposed rule for choosing a particular estimator in the family \([3]\), the parameter \(a\) is set according to a threshold 1 for the asymptotic standard deviation. The default value for 1 is 0.05 that is considered to be small enough, but other values can be chosen: the function `LBEa` described below illustrates the relation between \(a\) and 1 for a fixed number of tested hypotheses.

A particular value for \(a\) can also be directly set (without using the parameter 1). Choosing values of \(a\) less than 1 leads to use the identity function for transforming the p-values, that is to say the estimator \([2]\).

First we apply the function `LBE` by changing the upper bound for the asymptotic standard deviation, then, we apply `LBE` by arbitrarily setting \(a = 2\) and finally, we do not transform the p-values (by setting \(a = -1\)).
> LBE.res5 <- LBE(golub.pval, a=2, l=NA, plot.type="none")
> LBE.res5$a; LBE.res5$l; LBE.res5$pi0; LBE.res5$pi0.ci; LBE.res5$n.significant
[1] 2
[1] 0.02648321
[1] 0.5996953
[1] 0.0000000 0.6432563
[1] 1505

> LBE.res6 <- LBE(golub.pval, a=NA, l=0.1, plot.type="none")
> LBE.res6$a; LBE.res6$l; LBE.res6$pi0; LBE.res6$pi0.ci; LBE.res6$n.significant
[1] 4.025365
[1] 0.1000004
[1] 0.6015066
[1] 0.0000000 0.7659925
[1] 1505

> LBE.res7 <- LBE(golub.pval, a=-1, l=NA, plot.type="none")
> LBE.res7$a; LBE.res7$l; LBE.res7$pi0; LBE.res7$pi0.ci; LBE.res7$n.significant
[1] NA
[1] 0.006837937
[1] 0.6501887
[1] 0.0000000 0.6614361
[1] 1457

### 3.2 LBEplot

If plot.type="main", the function LBEplot, that is called by the main function LBE, displays the plot of the q-values versus the p-values together with the histogram of the p-values. The FDR and the numbers of significant and non significant genes are displayed in the legend.

If plot.type="multiple" (default value), the function LBEplot produces four plots: an histogram of the p-values, the plot of the q-values versus the p-values, the number of significant genes versus the q-values and the number of expected false positives by the number of significant genes.
The LBEa function is called by the main function LBE for choosing the greatest value of $a$ such that the upper bound of the asymptotic standard deviation is less than a threshold $l$. A plot illustrating the relation between $a$ and $l$ for a fixed number of tested hypotheses is displayed.

> LBEa(length(golub.pval), l=0.1)

[1] 4.025365
3.4 LBEsummary

The function *LBEsummary* is analogous to the function *summary* from the *qvalue* package. It reports an estimate and a confidence interval for the proportion of true null hypotheses and presents a table comparing p-values to q-values.

```
> LBEsummary(LBE.res)
```

Call:
LBE(pval = golub.pval)

pi0: 0.6022541
Confidence Interval (level=0.95): [0,0.6844972]

Cumulative number of significant calls:

  <1e-04 <0.001 <0.01 <0.025 <0.05 <0.1 <1
p-value  415  731  1287  1654  2053  2602  7129
q-value  236  415  858  1154  1505  2008  7129

3.5 LBEwrite

The LBEwrite function is analogous to the qwrite function from the qvalue package. It writes
the output of the function LBE to a file.

> LBEwrite(LBE.res)

4 Comparison with the qvalue package

The qvalue package contains functions for the estimation and presentation of the q-values
following the method introduced by Storey and Tibshirani [5]. While the default method
for estimating \( \pi_0 \) (in the qvalue function) relies on a smoothing method for estimating the
marginal density evaluated at one, LBE is based on the expectation of a transformation of the
p-values.

As regards to qvalue, the results of a simulation study [2] indicates good performances for
LBE. Moreover, the theoretical results we have obtained allow the calculation of a confidence
interval for \( \pi_0 \).

We compare here the two methods with the dataset from Golub et al. [3].

> library(qvalue)
> qvalue.res <- qvalue(golub.pval)
> summary(qvalue.res)

Call:
qvalue(p = golub.pval)

pi0: 0.5841632

Cumulative number of significant calls:

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<th></th>
<th>&lt;1e-04</th>
<th>&lt;0.001</th>
<th>&lt;0.01</th>
<th>&lt;0.025</th>
<th>&lt;0.05</th>
<th>&lt;0.1</th>
<th>&lt;1</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-value</td>
<td>415</td>
<td>731</td>
<td>1287</td>
<td>1654</td>
<td>2053</td>
<td>2602</td>
<td>7129</td>
</tr>
<tr>
<td>q-value</td>
<td>236</td>
<td>415</td>
<td>863</td>
<td>1163</td>
<td>1519</td>
<td>2037</td>
<td>7129</td>
</tr>
<tr>
<td>local FDR</td>
<td>160</td>
<td>291</td>
<td>571</td>
<td>734</td>
<td>921</td>
<td>1188</td>
<td>5758</td>
</tr>
</tbody>
</table>

> LBEsummary(LBE.res)

Call:
LBE(pval = golub.pval)

pi0: 0.6022541

Confidence Interval (level=0.95): [0.5844972]
Cumulative number of significant calls:

\[ <1e-04 <0.001 <0.01 <0.025 <0.05 <0.1 <1 \]

<table>
<thead>
<tr>
<th>p-value</th>
<th>415</th>
<th>731</th>
<th>1287</th>
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<tr>
<td>q-value</td>
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References


