Introduction to RBM package

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Contents

1 Overview

This document provides an introduction to the `RBM` package. The `RBM` package executes the resampling-based empirical Bayes approach using either permutation or bootstrap tests based on moderated t-statistics through the following steps.

- Firstly, the RBM package computes the moderated t-statistics based on the observed data set for each feature using the lmFit and eBayes function.
- Secondly, the original data are permuted or bootstrapped in a way that matches the null hypothesis to generate permuted or bootstrapped resamples, and the reference distribution is constructed using the resampled moderated t-statistics calculated from permutation or bootstrap resamples.
- Finally, the p-values from permutation or bootstrap tests are calculated based on the proportion of the permuted or bootstrapped moderated t-statistics that are as extreme as, or more extreme than, the observed moderated t-statistics.

Additional detailed information regarding resampling-based empirical Bayes approach can be found elsewhere (Li et al., 2013).

2 Getting started

The RBM package can be installed and loaded through the following R code.
Install the RBM package with:

```
> if (!requireNamespace("BiocManager", quietly=TRUE))
+     install.packages("BiocManager")
> BiocManager::install("RBM")
```

Load the RBM package with:

```
> library(RBM)
```

3 RBM_T and RBM_F functions

There are two functions in the RBM package: RBM_T and RBM_F. Both functions require input data in the matrix format with rows denoting features and columns denoting samples. RBM_T is used for two-group comparisons such as study designs with a treatment group and a control group. RBM_F can be used for more complex study designs such as more than two groups or time-course studies. Both functions need a vector for group notation, i.e., "1" denotes the treatment group and "0" denotes the control group. For the RBM_F function, a contrast vector need to be provided by users to perform pairwise comparisons between groups. For example, if the design has three groups (0, 1, 2), the aContrast parameter will be a vector such as ("X1-X0", "X2-X1", "X2-X0") to denote all pairwise comparisons. Users just need to add an extra "X" before the group labels to do the contrasts.

- Examples using the RBM_T function: normdata simulates a standardized gene expression data and unifdata simulates a methylation microarray data. The p-values from the RBM_T function could be further adjusted using the p.adjust function in the stats package through the Bejamini-Hochberg method.

```
> library(RBM)
> normdata <- matrix(rnorm(1000*6, 0, 1),1000,6)
> mydesign <- c(0,0,0,1,1,1)
> myresult <- RBM_T(normdata,mydesign,100,0.05)
> summary(myresult)

              Length Class  Mode
ordfit_t      1000   -none- numeric
ordfit_pvalue 1000   -none- numeric
ordfit_beta0  1000   -none- numeric
ordfit_beta1  1000   -none- numeric
permutation_p 1000   -none- numeric
bootstrap_p   1000   -none- numeric

> sum(myresult$permutation_p<=0.05)
```

```
[1] 11

> which(myresult$permutation_p<=0.05)

 [1]  16  47 262 324 353 399 519 686 835 860 940

> sum(myresult$bootstrap_p<=0.05)

[1] 22

> which(myresult$bootstrap_p<=0.05)

 [1]  14  70  86 143 183 190 255 285 302 429 438 481 511 577 640 661 746 811 831
[20] 835 882 953

> permutation_adjp <- p.adjust(myresult$permutation_p, "BH")
> sum(permutation_adjp<=0.05)

[1] 0

> bootstrap_adjp <- p.adjust(myresult$bootstrap_p, "BH")
> sum(bootstrap_adjp<=0.05)

[1] 0

> unifdata <- matrix(runif(1000*7,0.10, 0.95), 1000, 7)
> mydesign2 <- c(0,0,0, 1,1,1,1)
> myresult2 <- RBM_T(unifdata,mydesign2,100,0.05)
> sum(myresult2$permutatioin_p<=0.05)

[1] 0

> sum(myresult2$bootstrap_p<=0.05)

[1] 17

> which(myresult2$bootstrap_p<=0.05)

 [1]  72  98 127 174 179 181 196 371 431 526 633 809 814 846 899 921 978

> bootstrap2_adjp <- p.adjust(myresult2$bootstrap_p, "BH")
> sum(bootstrap2_adjp<=0.05)

[1] 1
```

- Examples using the `RBM_F` function: normdata_F simulates a standardized gene expression data and unifdata_F simulates a methylation microarray data. In both examples, we were interested in pairwise comparisons.

```
> normdata_F <- matrix(rnorm(1000*9,0,2), 1000, 9)
> mydesign_F <- c(0, 0, 0, 1, 1, 1, 2, 2, 2)
> aContrast <- c("X1-X0", "X2-X1", "X2-X0")
> myresult_F <- RBM_F(normdata_F, mydesign_F, aContrast, 100, 0.05)
> summary(myresult_F)

             Length Class  Mode
ordfit_t     3000   -none- numeric
ordfit_pvalue 3000   -none- numeric
ordfit_beta1  3000   -none- numeric
permutation_p 3000   -none- numeric
bootstrap_p   3000   -none- numeric

> sum(myresult_F$permutation_p[, 1]<=0.05)

[1] 60

> sum(myresult_F$permutation_p[, 2]<=0.05)

[1] 63

> sum(myresult_F$permutation_p[, 3]<=0.05)

[1] 72

> which(myresult_F$permutation_p[, 1]<=0.05)

 [1]   13   25   34   43   50  108  129  133  136  151  188  203  222  225  255
[16]  283  289  292  304  312  321  327  354  370  399  407  436  446  455  477
[31]  485  487  498  513  527  536  538  546  562  572  580  647  654  678  694
[46]  695  744  748  751  789  830  842  848  881  938  939  952  962  981 1000

> which(myresult_F$permutation_p[, 2]<=0.05)

 [1]  13  25  34  43  50  65  81 129 133 136 151 152 188 225 248 255 285 289 292
[20] 310 312 321 327 332 354 370 407 436 446 454 458 477 487 493 498 513 527 529
[39] 536 538 546 580 589 608 638 654 678 694 695 744 748 751 761 781 789 842 848
[58] 881 938 939 952 962 981

> which(myresult_F$permutation_p[, 3]<=0.05)

 [1]  13  25  26  34  43  50  65  67  81 129 133 136 143 151 188 198 203 222 225
[20] 272 283 289 292 304 312 321 327 332 346 354 362 370 407 446 454 455 477 487
[39] 498 513 527 531 536 538 546 562 572 580 608 647 654 678 685 694 695 744 748
[58] 761 781 789 830 842 848 870 881 906 938 939 952 959 962 981

> con1_adjp <- p.adjust(myresult_F$permutation_p[, 1], "BH")
> sum(con1_adjp<=0.05/3)
```

```
[1] 13

> con2_adjp <- p.adjust(myresult_F$permutation_p[, 2], "BH")
> sum(con2_adjp<=0.05/3)

[1] 13

> con3_adjp <- p.adjust(myresult_F$permutation_p[, 3], "BH")
> sum(con3_adjp<=0.05/3)

[1] 14

> which(con2_adjp<=0.05/3)

 [1]  13  43  50 136 188 289 354 477 538 546 580 678 952

> which(con3_adjp<=0.05/3)

 [1]  13  25  34  50 136 188 203 289 487 538 580 695 952 962

> unifdata_F <- matrix(runif(1000*18, 0.15, 0.98), 1000, 18)
> mydesign2_F <- c(rep(0, 6), rep(1, 6), rep(2, 6))
> aContrast <- c("X1-X0", "X2-X1", "X2-X0")
> myresult2_F <- RBM_F(unifdata_F, mydesign2_F, aContrast, 100, 0.05)
> summary(myresult2_F)

             Length Class  Mode
ordfit_t     3000   -none- numeric
ordfit_pvalue 3000   -none- numeric
ordfit_beta1  3000   -none- numeric
permutation_p 3000   -none- numeric
bootstrap_p   3000   -none- numeric

> sum(myresult2_F$bootstrap_p[, 1]<=0.05)

[1] 45

> sum(myresult2_F$bootstrap_p[, 2]<=0.05)

[1] 54

> sum(myresult2_F$bootstrap_p[, 3]<=0.05)

[1] 33

> which(myresult2_F$bootstrap_p[, 1]<=0.05)
```

```
 [1]  72 102 105 125 134 152 154 185 269 283 290 378 410 437 468 470 485 495 534
[20] 543 556 638 648 662 664 715 716 731 739 756 773 783 841 880 894 895 906 908
[39] 934 935 938 960 972 973 974

> which(myresult2_F$bootstrap_p[, 2]<=0.05)

 [1]  52  55  72  94 102 109 119 120 125 134 152 185 217 233 247 254 269 270 283
[20] 364 378 410 468 470 495 534 543 564 574 638 648 656 662 664 713 715 716 731
[39] 756 773 783 808 816 841 880 894 895 906 934 935 938 960 973 974

> which(myresult2_F$bootstrap_p[, 3]<=0.05)

 [1]  72 102 105 125 152 185 247 269 283 290 364 378 410 470 495 513 534 543 648
[20] 656 662 664 715 716 756 783 822 880 894 895 938 960 974

> con21_adjp <- p.adjust(myresult2_F$bootstrap_p[, 1], "BH")
> sum(con21_adjp<=0.05/3)

[1] 7

> con22_adjp <- p.adjust(myresult2_F$bootstrap_p[, 2], "BH")
> sum(con22_adjp<=0.05/3)

[1] 3

> con23_adjp <- p.adjust(myresult2_F$bootstrap_p[, 3], "BH")
> sum(con23_adjp<=0.05/3)

[1] 2
```

4 Ovarian cancer methylation example using the RBM_T function

Two-group comparisons are the most common contrast in biological and biomedical field. The ovarian cancer methylation example is used to illustrate the application of RBM_T in identifying differentially methylated loci. The ovarian cancer methylation example is taken from the gemone-wide DNA methylation profiling of United Kingdom Ovarian Cancer Population Study (UKOPS). This study used Illumina Infinium 27k Human DNA methylation Beadchip v1.2 to obtain DNA methylation profiles on over 27,000 CpGs in whole blood cells from 266 ovarian cancer women and 274 age-matched healthy controls. The data are downloaded from the NCBI GEO website with access number GSE19711. For illutration purpose, we chose the first 1000 loci in 8 randomly selected women with 4 ovariance cancer cases (pre-treatment) and 4 healthy controls. The following codes show the process of generating significant differential DNA methylation loci using the RBM_T function and presenting the results for further validation and investigations.

```
> system.file("data", package = "RBM")

[1] "/private/tmp/Rtmp1KcFNJ/Rinstf574126294b2/RBM/data"
```

```
> data(ovarian_cancer_methylation)
> summary(ovarian_cancer_methylation)

        IlmnID         Beta          exmdata2[, 2]     exmdata3[, 2]
 cg00000292:  1   Min.   :0.01058   Min.   :0.01187   Min.   :0.009103
 cg00002426:  1   1st Qu.:0.04111   1st Qu.:0.04407   1st Qu.:0.041543
 cg00003994:  1   Median :0.08284   Median :0.09531   Median :0.087042
 cg00005847:  1   Mean   :0.27397   Mean   :0.28872   Mean   :0.283729
 cg00006414:  1   3rd Qu.:0.52135   3rd Qu.:0.59032   3rd Qu.:0.558575
 cg00007981:  1   Max.   :0.97069   Max.   :0.96937   Max.   :0.970155
 (Other)   :994                     NA's   :4
 exmdata4[, 2]     exmdata5[, 2]     exmdata6[, 2]     exmdata7[, 2]
 Min.   :0.01019   Min.   :0.01108   Min.   :0.01937   Min.   :0.01278
 1st Qu.:0.04092   1st Qu.:0.04059   1st Qu.:0.05060   1st Qu.:0.04260
 Median :0.09042   Median :0.08527   Median :0.09502   Median :0.09362
 Mean   :0.28508   Mean   :0.28482   Mean   :0.27348   Mean   :0.27563
 3rd Qu.:0.57502   3rd Qu.:0.57300   3rd Qu.:0.52099   3rd Qu.:0.52240
 Max.   :0.96658   Max.   :0.97516   Max.   :0.96681   Max.   :0.95974
                   NA's   :1
 exmdata8[, 2]
 Min.   :0.01357
 1st Qu.:0.04387
 Median :0.09282
 Mean   :0.28679
 3rd Qu.:0.57217
 Max.   :0.96268

> ovarian_cancer_data <- ovarian_cancer_methylation[, -1]
> label <- c(1, 1, 0, 0, 1, 1, 0, 0)
> diff_results <- RBM_T(aData=ovarian_cancer_data, vec_trt=label, repetition=100, alpha=0.05)
> summary(diff_results)

             Length Class  Mode
ordfit_t     1000   -none- numeric
ordfit_pvalue 1000   -none- numeric
ordfit_beta0  1000   -none- numeric
ordfit_beta1  1000   -none- numeric
permutation_p 1000   -none- numeric
bootstrap_p   1000   -none- numeric

> sum(diff_results$ordfit_pvalue<=0.05)

[1] 45

> sum(diff_results$permutation_p<=0.05)

[1] 38
```

```
> sum(diff_results$bootstrap_p<=0.05)

[1] 51

> ordfit_adjp <- p.adjust(diff_results$ordfit_pvalue, "BH")
> sum(ordfit_adjp<=0.05)

[1] 0

> perm_adjp <- p.adjust(diff_results$permutation_p, "BH")
> sum(perm_adjp<=0.05)

[1] 4

> boot_adjp <- p.adjust(diff_results$bootstrap_p, "BH")
> sum(boot_adjp<=0.05)

[1] 0

> diff_list_perm <- which(perm_adjp<=0.05)
> diff_list_boot <- which(boot_adjp<=0.05)
> sig_results_perm <- cbind(ovarian_cancer_methylation[diff_list_perm, ], diff_results$ordfit_t[
> print(sig_results_perm)

        IlmnID       Beta exmdata2[, 2] exmdata3[, 2] exmdata4[, 2]
19  cg00016968 0.80628480            NA    0.81440820    0.83623180
259 cg00234961 0.04192170    0.04321576    0.05707140    0.05327565
848 cg00826384 0.05721674    0.05612171    0.06644259    0.06358381
851 cg00830029 0.58362500    0.59397870    0.64739610    0.67269640
    exmdata5[, 2] exmdata6[, 2] exmdata7[, 2] exmdata8[, 2]
19     0.80831380    0.73306440    0.82968340    0.84917800
259    0.04030003    0.03996053    0.05086962    0.05445672
848    0.05230160    0.06119713    0.06542751    0.06240686
851    0.50820240    0.34657470    0.66276570    0.64634510
    diff_results$ordfit_t[diff_list_perm]
19                              -2.446404
259                             -4.052697
848                             -2.314412
851                             -2.841244
    diff_results$permutation_p[diff_list_perm]
19                                       0
259                                      0
848                                      0
851                                      0

> sig_results_boot <- cbind(ovarian_cancer_methylation[diff_list_boot, ], diff_results$ordfit_t[
> print(sig_results_boot)
```

```
 [1] IlmnID
 [2] Beta
 [3] exmdata2[, 2]
 [4] exmdata3[, 2]
 [5] exmdata4[, 2]
 [6] exmdata5[, 2]
 [7] exmdata6[, 2]
 [8] exmdata7[, 2]
 [9] exmdata8[, 2]
[10] diff_results$ordfit_t[diff_list_boot]
[11] diff_results$bootstrap_p[diff_list_boot]
<0 rows> (or 0-length row.names)
```