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] 27

> which(myresult$permutation_p<=0.05)

 [1]  69  87  98 129 131 144 203 215 278 443 470 502 569 582 583 587 627 635 640
[20] 731 799 803 827 894 917 931 991

> sum(myresult$bootstrap_p<=0.05)

[1] 2

> which(myresult$bootstrap_p<=0.05)

[1] 164 340

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

[1] 1

> 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] 13

> which(myresult2$bootstrap_p<=0.05)

 [1]  30 121 139 163 232 447 506 631 809 838 854 986 988

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

[1] 0
```

- 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] 48

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

[1] 68

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

[1] 56

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

 [1]  11  39  40  51 105 204 206 231 255 259 260 291 293 297 316 366 402 421 445
[20] 496 500 502 530 533 551 589 653 655 702 711 713 727 776 809 811 826 873 874
[39] 882 903 908 913 919 932 950 969 978 979

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

 [1]  11  22  39  51 100 105 182 206 211 231 255 259 260 266 291 297 302 327 347
[20] 356 358 366 402 421 445 472 496 500 502 519 530 533 543 559 565 589 594 619
[39] 633 634 638 653 655 702 711 713 727 749 776 789 804 809 811 826 849 869 873
[58] 874 882 903 913 914 924 932 935 950 969 978

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

 [1]  11  39  40  51 105 182 195 206 231 255 259 260 266 291 293 297 327 347 358
[20] 366 402 421 445 500 502 519 530 543 565 589 594 633 653 655 677 693 702 711
[39] 713 727 776 789 809 811 826 869 873 874 882 903 919 932 950 969 978 979

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

[1] 10
```

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

[1] 11

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

[1] 12

> which(con2_adjp<=0.05/3)

 [1]  39 105 327 402 589 653 702 713 727 874 978

> which(con3_adjp<=0.05/3)

 [1]  39 105 206 402 502 589 653 711 727 776 903 978

> 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] 57

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

[1] 45

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

[1] 63

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

 [1]   3   4  12  17  27  31  49  72  93  95  99 169 175 177 200 248 263 288 296
[20] 311 316 333 349 368 378 408 432 445 453 471 487 506 510 544 554 618 624 637
[39] 655 666 699 702 704 716 732 745 758 829 836 873 874 891 907 912 915 932 969
```

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

 [1]  12  17  31  49  72  93  95  99 135 175 200 288 296 311 316 368 378 408 432
[20] 487 510 540 544 613 618 624 637 655 666 699 702 704 706 716 732 774 829 836
[39] 864 874 891 911 915 932 969

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

 [1]   3   4  12  17  27  49  72  93  95  99 135 169 175 177 200 248 263 288 296
[20] 311 316 348 349 368 378 408 432 445 487 506 510 544 554 593 613 618 624 633
[39] 634 637 655 666 699 702 704 716 732 745 774 799 829 836 839 850 873 874 891
[58] 907 912 915 929 932 969

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

[1] 6

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

[1] 1

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

[1] 11
```

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] "/tmp/RtmpIlrW3r/Rinst41d21cd8dfb6/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] 50

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

```
[1] 54

> 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] 5

> 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]
245 cg00224508 0.04479948    0.04972043    0.04152814    0.04189373
280 cg00260778 0.64319890    0.60488960    0.56735060    0.53150910
627 cg00612467 0.04777553    0.03783457    0.05380982    0.05582291
764 cg00730260 0.90471270    0.90542290    0.91002680    0.91258610
    exmdata5[, 2] exmdata6[, 2] exmdata7[, 2] exmdata8[, 2]
245    0.04208405    0.05284988    0.03775905    0.03955271
280    0.61920530    0.61925200    0.46753250    0.55632410
627    0.04740551    0.05332965    0.05775211    0.05579710
764    0.90575890    0.88760470    0.90756300    0.90946790
    diff_results$ordfit_t[diff_list_perm]
245                              1.962457
280                              4.170347
627                             -2.239498
764                             -1.808081
    diff_results$permutation_p[diff_list_perm]
245                                         0
280                                         0
627                                         0
764                                         0

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

```
        IlmnID       Beta exmdata2[, 2] exmdata3[, 2] exmdata4[, 2]
95  cg00081975 0.03633894    0.04975194    0.06024723    0.05598723
259 cg00234961 0.04192170    0.04321576    0.05707140    0.05327565
280 cg00260778 0.64319890    0.60488960    0.56735060    0.53150910
833 cg00814580 0.09348613    0.09619816    0.12010440    0.11534240
911 cg00888479 0.07388961    0.07361080    0.10149800    0.09985076
    exmdata5[, 2] exmdata6[, 2] exmdata7[, 2] exmdata8[, 2]
95     0.04561792    0.05115624    0.06068253    0.06168212
259    0.04030003    0.03996053    0.05086962    0.05445672
280    0.61920530    0.61925200    0.46753250    0.55632410
833    0.09577040    0.11598850    0.12860890    0.14111200
911    0.08633986    0.06765189    0.09070268    0.12417730
    diff_results$ordfit_t[diff_list_boot]
95                              -3.252063
259                             -4.052697
280                              4.170347
833                             -3.428319
911                             -3.621731
    diff_results$bootstrap_p[diff_list_boot]
95                                       0
259                                      0
280                                      0
833                                      0
911                                      0
```