

Introduction to RBM package

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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 Benjamini-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] 21

> which(myresult$permutation_p<=0.05)

[1] 9 40 196 211 272 328 343 349 352 391 447 493 641 649 668 704 748 773 816
[20] 859 945

> sum(myresult$bootstrap_p<=0.05)

[1] 7

> which(myresult$bootstrap_p<=0.05)

[1] 256 391 445 459 470 499 695

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

[1] 2

> bootstrap_adj_p <- p.adjust(myresult$bootstrap_p, "BH")
> sum(bootstrap_adj_p<=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] 10

> which(myresult2$bootstrap_p<=0.05)

[1] 53 66 213 470 490 661 794 821 994 995

> bootstrap2_adj_p <- p.adjust(myresult2$bootstrap_p, "BH")
> sum(bootstrap2_adj_p<=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] 50

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

[1] 43

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

[1] 53

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

[1]  37  66  94 122 123 163 166 167 170 223 235 263 277 292 294 325 340 364 368
[20] 411 414 463 467 485 489 492 515 549 599 621 631 671 674 677 682 685 714 725
[39] 734 780 789 794 860 870 879 912 942 958 977 994

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

[1]  37  66  94 113 123 142 166 235 270 277 292 334 362 368 407 411 437 463 467
[20] 489 515 549 575 589 599 621 625 631 671 674 677 685 725 734 780 794 807 860
[39] 870 891 912 942 958

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

[1]  37  39  66  94 113 116 122 142 187 223 277 292 324 364 367 368 376 403 411
[20] 463 467 489 507 515 549 570 575 589 599 621 625 631 654 671 674 677 682 685
[39] 734 774 780 794 807 809 837 860 869 870 879 891 912 958 994

> 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] 5

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

[1] 5

> which(con2_adjp<=0.05/3)

[1] 94 463 489 674 677

> which(con3_adjp<=0.05/3)

[1] 94 489 621 682 685

> 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] 60

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

[1] 53

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

[1] 49

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

[1] 22 32 47 48 55 81 84 96 107 126 148 174 194 202 211 220 237 242 243
[20] 249 263 264 290 302 327 333 335 339 359 396 398 428 451 460 545 547 549 599
[39] 616 626 640 690 703 706 708 712 733 740 825 842 849 852 861 905 911 931 939
[58] 942 975 997

```

```

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

[1] 47 48 55 57 96 107 126 148 167 174 194 202 220 237 242 249 285 302 320
[20] 327 335 359 395 396 405 428 430 451 522 545 547 599 616 626 640 643 690 708
[39] 712 733 740 754 771 789 825 849 852 905 911 931 942 967 997

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

[1] 32 47 57 96 107 124 126 148 194 220 237 242 243 249 285 290 305 320 327
[20] 333 335 396 430 451 545 547 599 626 638 640 643 690 708 712 740 754 771 789
[39] 825 849 852 861 905 911 931 939 942 967 997

> con21_adj_p <- p.adjust(myresult2_F$bootstrap_p[, 1], "BH")
> sum(con21_adj_p<=0.05/3)

[1] 3

> con22_adj_p <- p.adjust(myresult2_F$bootstrap_p[, 2], "BH")
> sum(con22_adj_p<=0.05/3)

[1] 5

> con23_adj_p <- p.adjust(myresult2_F$bootstrap_p[, 3], "BH")
> sum(con23_adj_p<=0.05/3)

[1] 3

```

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 genome-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 illustration purpose, we chose the first 1000 loci in 8 randomly selected women with 4 ovarian 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/RtmpEdjKdh/Rinst17551bd43c6db/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] 67

> sum(diff_results$bootstrap_p<=0.05)

```

```
[1] 53
```

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

```
[1] 0
```

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

```
[1] 5
```

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

```
[1] 0
```

```
> diff_list_perm <- which(perm_adj_p<=0.05)
> diff_list_boot <- which(boot_adj_p<=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
245	cg00224508	0.04479948	0.04972043	0.04152814	0.04189373
450	cg00432979	0.03681359	0.04515700	0.04374394	0.03683598
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]	
19	0.80831380	0.73306440	0.82968340	0.84917800	
245	0.04208405	0.05284988	0.03775905	0.03955271	
450	0.04419125	0.04409653	0.02839263	0.03410020	
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]				
19	-2.446404				
245	1.962457				
450	1.546114				
627	-2.239498				
764	-1.808081				
	diff_results\$permutation_p[diff_list_perm]				
19	0				
245	0				
450	0				
627	0				
764	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)

```