

# miRtest v. 1.0 Package Vignette

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## 1 Introduction

High-throughput measurements of gene expression are gaining popularity. So are microRNA analyses. The ‘miRtest’ package (Artmann *et al.*, 2011) intends to help researches find regulated expressed miRNAs between two groups.

‘miRtest’ tries to improve power when testing for differentially regulated miRNAs by incorporation of their regulated gene sets’ expression data.

miRNA-wise testing is done with the linear models implemented in the ‘limma’ package (Smyth, 2004). For gene set testing, different procedures can be chosen from: the self-contained tests ‘globaltest’ (Goeman *et al.*, 2004), ‘GlobalAncova’ (Mansmann and Meister, 2005; Hummel *et al.*, 2008), ‘RepeatedHighDim’ (Jung *et al.*, 2011; Brunner, 2009), the competitive tests ‘ROAST’ (Wu *et al.*, 2010) and ‘Romer’ (Majewski *et al.*, 2010) as well as non-rotation enrichment tests.

## 2 Simple Example

The main function of ‘miRtest’ is ‘miR.test’. It requires the user to supply an expression matrix  $\mathbf{X}$  of miRNAs with miRNAs in its rows and microarray samples in its columns. Additionally, the procedures require an analogous matrix  $\mathbf{Y}$  of mRNA expression values. Finally, a data.frame  $\mathbf{A}$  is necessary: it defines which mRNAs are attacked by which miRNA. To begin with, we will generate random expression data: miRNA expression matrix  $\mathbf{X}$ , mRNA expression matrix  $\mathbf{Y}$  and an allocation data.frame  $\mathbf{A}$ .

```
> set.seed(1)
> X = rnorm(24)
> dim(X) = c(3, 8)
> rownames(X) = 1:3
```

In this synthetic experiment, 8 microarray replicates are present with three miRNAs on each. Additionally, we need a corresponding matrix  $\mathbf{Y}$  for mRNAs. Here we assume we have 20 mRNAs and 10 microarray replicates:

```
> Y = rnorm(200)
> dim(Y) = c(20, 10)
> rownames(Y) = 1:20
```

Now we need to define what we want to test for. We shall concentrate on two-group testing, i. e. the search for miRNAs differentially expressed between two groups 1 and 2. For other designs see Section 5. Let’s say both groups are of equal sample size in miRNA and mRNA microarrays:

```
> group.miRNA = factor(c(1, 1, 1, 1, 2, 2, 2, 2))
> group.mRNA = factor(c(1, 1, 1, 1, 1, 2, 2, 2, 2, 2))
```

Next, we need the allocation information. In most databases it is provided as a data.frame  $\mathbf{A}$ , where the first column contains mRNAs and the second miRNAs. Each row of  $\mathbf{A}$  indicates which mRNA is targeted by which miRNA. Let’s say that miRNA 1 has nine target genes and miRNA 2 the remaining ones. The gene set of miRNA 3 will be empty.

```
> library(miRtest)
> miR = c(rep(1, 9), c(rep(2, 8)))
> mRNAs = 1:17
> A = data.frame(mRNAs, miR)
> A
```

	mRNAs	miR
1	1	1
2	2	1
3	3	1

4	4	1
5	5	1
6	6	1
7	7	1
8	8	1
9	9	1
10	10	2
11	11	2
12	12	2
13	13	2
14	14	2
15	15	2
16	16	2
17	17	2

Finally, the function ‘miR.test’ is called which does the testing.

```
> set.seed(1)
> P = miR.test(X, Y, A, group.miRNA, group.mRNA)
> P
```

```
      miRtest
1 0.7208223
2 0.3157580
3      NA
```

Note that for the empty gene set ‘NA’ was returned.

### 3 Choice of Gene Set Tests

The ‘gene.set.test’ argument in `miR.test` takes a vector of strings. These are the gene set tests that shall be applied. The default is the ‘romer’ test as it is competitive and compensates for inter-gene correlations. The different gene set tests available are:

Test	Name in <code>miR.test</code>
<b>Self-contained</b>	
‘globaltest’ (Goeman <i>et al.</i> , 2004)	”globaltest”
‘GlobalAncova’ (Mansmann and Meister, 2005; Hummel <i>et al.</i> , 2008)	”GA”
‘RepeatedHighDim’ (Brunner, 2009)	”RHD”
<b>Competitive</b>	
Kolm. Smirnov test on gene ranks	”KS”
Wilcoxon test on gene ranks	”W”
Fisher’s exact test on gene ranks with 5 % FDR threshold	”Fisher”
‘ROAST’ (Wu <i>et al.</i> , 2010)	”roast”
‘romer’ (Majewski <i>et al.</i> , 2010)	”romer”

#### 3.1 Faster Algorithm

The specification of other gene set tests in `miR.test` is therefore rather simple. To obtain faster results than with the default ‘romer’ rotation test, the Wilcoxon two-sample test based on gene ranks is recommended:

```
> P.gsWilcox = miR.test(X, Y, A, group.miRNA, group.mRNA, gene.set.tests = "W")
> P.gsWilcox
```

```
      miRtest
1 0.8520745
2 0.3297708
3      NA
```

## 4 Other Input Formats of Allocation Data

To make ‘miR.test’ run faster one can specify an allocation matrix instead of the allocation data.frame. Its columns stand for the miRNAs and its rows for the mRNAs. If a mRNA is a target of a miRNA, the corresponding entry is 1, else it is 0. An easy way to generate allocation matrices is the ‘generate.A’ function:

```
> A = generate.A(A, X = X, Y = Y, verbose = FALSE)
> A
```

```
      1 2 3
1  1 0 0
2  1 0 0
3  1 0 0
4  1 0 0
5  1 0 0
6  1 0 0
7  1 0 0
8  1 0 0
9  1 0 0
10 0 1 0
11 0 1 0
12 0 1 0
13 0 1 0
14 0 1 0
15 0 1 0
16 0 1 0
17 0 1 0
18 0 0 0
19 0 0 0
20 0 0 0
```

To use the allocation matrix, we need to set ‘allocation.matrix=TRUE’ in ‘miR.test’:

```
> set.seed(1)
> P = miR.test(X, Y, A, group.miRNA, group.mRNA, allocation.matrix = TRUE)
> P
```

```
      miRtest
1 0.7208223
2 0.3157580
3      NA
```

## 5 Other Designs than Two-Group Design

Primarily, ‘miRtest’ has been designed for two-group comparisons. However, ‘miRtest’ accepts design matrices as used in ‘limma’ (Smyth, 2004). The only limitation is that ‘miRtest’ takes the second column from ‘limma’s ‘eBayes’ function to calculate final  $p$ -values. This already allows designs including

- covariables and
- continuous group/response vectors.

Other designs will be implemented in future versions. Regard the following example which shows how to use ‘miRtest’ on such designs. First we create the design matrices

```
> group.miRNA = 1:8
> group.mRNA = 1:10
> covariable.miRNA = factor(c(1, 2, 3, 4, 1, 2, 3, 4))
> covariable.mRNA = factor(c(1, 2, 3, 4, 5, 1, 2, 3, 4, 5))
> library(limma)
> design.miRNA = model.matrix(~group.miRNA + covariable.miRNA)
> design.mRNA = model.matrix(~group.mRNA + covariable.mRNA)
```

which then we use in ‘miR.test’

```
> P = miR.test(X, Y, A, design.miRNA = design.miRNA, design.mRNA = design.mRNA,
+ allocation.matrix = TRUE)
> P
```

```
miRtest
1 0.5797948
2 0.4981197
3          NA
```

Note that so far this works only with the competitive gene set tests and ‘ROAST’.

## References

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