miRtest v. 1.3 Package Vignette

Stephan Artmann <stephan-artmann@gmx.net>,
Klaus Jung,
Tim Beißbarth

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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.*, 2012) intends to help researchers find differentially 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.*, sub; Brunner, 2009), the rotation 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 $\boldsymbol{X}$ of miRNAs with miRNAs in its rows and microarray samples in its columns. Additionally, the procedures require an analogous matrix $\boldsymbol{Y}$ of mRNA expression values. Finally, a data.frame $\boldsymbol{A}$ is necessary: it defines which mRNAs are attacked by which miRNA. To begin with, we will generate random expression data: miRNA expression matrix $\boldsymbol{X}$, mRNA expression matrix $\boldsymbol{Y}$ and an allocation data.frame $\boldsymbol{A}$. 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:
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Test	**Name in miR.test**
Self-contained	
'globaltest' (Goeman *et˜al.*, 2004)	"globaltest"
'GlobalAncova'	"GA"
(Mansmann and Meister, 2005; Hummel *et˜al.*, 2008)	
'RepeatedHighDim' (Brunner, 2009)	"RHD"
Competitive	
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 *et˜al.*, 2010)	"roast"
'romer' (Majewski *et˜al.*, 2010)	"romer"

3.1 Faster Algorithm

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:

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 P now contains the Benjamini-Hochberg-adjusted p-values for the miRNAs we investigated. Note that for competitive testing the entire mRNA-data would be necessary, while only a subset of mRNAs was used here.

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