

miRNAAtap example use

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May 4, 2016

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1 Introduction

`miRNA` package is designed to facilitate implementation of workflows requiring miRNA prediction. Aggregation of commonly used prediction algorithm outputs in a way that improves on performance of every single one of them on their own when compared against experimentally derived targets. microRNA (miRNA) is a 18-22nt long single strand that binds with RISC (RNA induced silencing complex) and targets mRNAs effectively reducing their translation rates.

Targets are aggregated from 4 most commonly cited prediction algorithms: DIANA (Maragkakis et al., 2011), Miranda (Enright et al., 2003), PicTar (Lall et al., 2006) and TargetScan (Friedman et al., 2009).

Programmatic access to sources of data is crucial when streamlining the workflow of our analysis, this way we can run similar analysis for multiple input miRNAs or any other parameters. Not only does it allow us to obtain predictions from multiple sources straight into R but also through aggregation of sources it improves the quality of predictions.

Finally, although direct predictions from all sources are only available for *Homo sapiens* and *Mus musculus*, this package includes an algorithm that allows to translate target genes to other speices (currently only *Rattus norvegicus*) using homology information where direct targets are not available.

2 Installation

This section briefly describes the necessary steps to get `miRNA` running on your system. We assume that the user has the R program (see the R project at <http://www.r-project.org>) already installed and is familiar with it. You will need to have R 3.2.0 or later to be able to install and run `miRNA`. The `miRNA` package is available from the Bioconductor repository at <http://www.bioconductor.org> To be able to install the package one needs first to install the core Bioconductor packages. If you have already installed Bioconductor packages on your system then you can skip the two lines below.

```
> source("http://bioconductor.org/biocLite.R")
> biocLite()
```

Once the core Bioconductor packages are installed, we can install the `miRNA` and accompanying database `miRNA.db` package by

```
> source("http://bioconductor.org/biocLite.R")
> biocLite("miRNA")
> biocLite("miRNA.db")
```

3 Workflow

This section explains how `miRNA` package can be integrated in the workflow aimed at predicting which processes can be regulated by a given microRNA.

In this example workflow we'll use `miRNAatap` as well as another Bioconductor package `topGO` together with Gene Ontology (GO) annotations. In case we don't have `topGO` or GO annotations on our machine we need to install them first:

```
> source("http://bioconductor.org/biocLite.R")
> biocLite("topGO")
> biocLite("org.Hs.eg.db")
```

Then, let's load the required libraries

```
> library(miRNAatap)
> library(topGO)
> library(org.Hs.eg.db)
```

Now we can start the analysis. First, we will obtain predicted targets for human miRNA *miR-10b*

```
> mir = 'miR-10b'
> predictions = getPredictedTargets(mir, species = 'hsa',
+                                 method = 'geom', min_src = 2)
```

Let's inspect the top of the prediction list.

```
> head(predictions)
```

	source_1	source_2	source_3	source_4	rank_product	rank_final
6095	7	7.0	16	NA	3.073624	1
9612	137	10.0	3	8	3.366456	2
64641	16	22.0	5	NA	4.024540	3
627	121	17.5	1	NA	4.280422	4
253559	88	3.0	11	NA	4.755662	5
8013	51	1.0	86	NA	5.456342	6

We are using *geometric mean* aggregation method as it proves to perform best when tested against experimental data from MirBase (Griffiths-Jones et al., 2008).

We can compare it to the top of the list of the output of *mimumum* method:

```
> predictions_min = getPredictedTargets(mir, species = 'hsa',
+                                     method = 'min', min_src = 2)
> head(predictions_min)
```

	source_1	source_2	source_3	source_4	rank_product	rank_final
627	121	17.5	1	NA	1	2.5
8013	51	1.0	86	NA	1	2.5
23114	1	163.0	222	NA	1	2.5
65078	NA	65.5	97	1	1	2.5
1385	NA	2.0	161	NA	2	6.5
7022	105	240.5	2	73	2	6.5

Where predictions for rat genes are not available we can obtain predictions for mouse genes and translate them into rat genes through homology. The operation happens automatically if we specify species as `rno` (for *Rattus norvegicus*)

```
> predictions_rat = getPredictedTargets(mir, species = 'rno',
+                                     method = 'geom', min_src = 2)
```

Now we can use the ranked results as input to GO enrichment analysis. For that we will use our initial prediction for human *miR-10b*

```
> rankedGenes = predictions[, 'rank_product']
> selection = function(x) TRUE
> # we do not want to impose a cut off, instead we are using rank information
> allGO2genes = annFUN.org(whichOnto='BP', feasibleGenes = NULL,
+                          mapping="org.Hs.eg.db", ID = "entrez")
> GOdata = new('topGOdata', ontology = 'BP', allGenes = rankedGenes,
+             annot = annFUN.GO2genes, GO2genes = allGO2genes,
+             geneSel = selection, nodeSize=10)
```

In order to make use of the rank information we will use Kolomonogorov Smirnov (K-S) test instead of Fisher exact test which is based only on counts.

```
> results.ks = runTest(GOdata, algorithm = "classic", statistic = "ks")
> results.ks
```

Description:

Ontology: BP

'classic' algorithm with the 'ks' test

635 GO terms scored: 3 terms with p < 0.01

Annotation data:

Annotated genes: 279

Significant genes: 279

Min. no. of genes annotated to a GO: 10

Nontrivial nodes: 635

We can view the most enriched GO terms (and potentially feed them to further steps in our workflow)

```
> allRes = GenTable(GOdata, KS = results.ks, orderBy = "KS", topNodes = 20)
> allRes[, c('GO.ID', 'Term', 'KS')]
```

	GO.ID	Term	KS
1	GO:0042692	muscle cell differentiation	0.0033
2	GO:0006974	cellular response to DNA damage stimulus	0.0050
3	GO:0051146	striated muscle cell differentiation	0.0071
4	GO:0006352	DNA-templated transcription, initiation	0.0187
5	GO:0006367	transcription initiation from RNA polyme...	0.0187
6	GO:0070997	neuron death	0.0207

7	GO:1901214	regulation of neuron death	0.0207
8	GO:0006357	regulation of transcription from RNA pol...	0.0255
9	GO:0045944	positive regulation of transcription fro...	0.0277
10	GO:0010941	regulation of cell death	0.0285
11	GO:0090304	nucleic acid metabolic process	0.0296
12	GO:0050877	neurological system process	0.0316
13	GO:0061061	muscle structure development	0.0318
14	GO:0007417	central nervous system development	0.0368
15	GO:0006139	nucleobase-containing compound metabolic...	0.0374
16	GO:0016070	RNA metabolic process	0.0379
17	GO:0007548	sex differentiation	0.0386
18	GO:0008406	gonad development	0.0386
19	GO:0045137	development of primary sexual characteri...	0.0386
20	GO:0048513	animal organ development	0.0397

For more details about GO analysis refer to `topGO` package vignette (Alexa and Rahnenfuhrer, 2010).

Finally, we can use our predictions in a similar way for pathway enrichment analysis based on KEGG (Kanehisa and Goto, 2000), for example using Bioconductor's `KEGGprofile` (Zhao, 2012).

4 Session Information

- R version 3.3.0 RC (2016-04-25 r70549), x86_64-w64-mingw32
- Locale: LC_COLLATE=C, LC_CTYPE=English_United States.1252, LC_MONETARY=English_United States.1252, LC_NUMERIC=C, LC_TIME=English_United States.1252
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: AnnotationDbi 1.34.0, Biobase 2.32.0, BiocGenerics 0.18.0, GO.db 3.3.0, IRanges 2.6.0, S4Vectors 0.10.0, SparseM 1.7, graph 1.50.0, miRNAatap 1.6.0, miRNAatap.db 0.99.7, org.Hs.eg.db 3.3.0, topGO 2.24.0
- Loaded via a namespace (and not attached): DBI 0.4, RSQLite 1.0.0, Rcpp 0.12.4.5, chron 2.3-47, grid 3.3.0, gsubfn 0.6-6, lattice 0.20-33, magrittr 1.5, matrixStats 0.50.2, plyr 1.8.3, proto 0.3-10, sqldf 0.4-10, stringi 1.0-1, stringr 1.0.0, tools 3.3.0

References

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- Zhao, S. (2012). *KEGGprofile: An annotation and visualization package for multi-types and multi-groups expression data in KEGG pathway*. R package version 1.6.1.