

miRNAtap example use

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1 Introduction

miRNAtap 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 5 most commonly cited prediction algorithms: DIANA (Maragkakis et al., 2011), Miranda (Enright et al., 2003), PicTar (Lall et al., 2006), TargetScan (Friedman et al., 2009), and miRDB (Wong and Wang, 2015).

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 miRNAtap 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 miRNAtap. The miRNAtap 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.

```
> if (!requireNamespace("BiocManager", quietly=TRUE))
+   install.packages("BiocManager")
> BiocManager::install()
```

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

```
> if (!requireNamespace("BiocManager", quietly=TRUE))
+   install.packages("BiocManager")
> BiocManager::install("miRNAtap")
> BiocManager::install("miRNAtap.db")
```

3 Workflow

This section explains how `miRNAatap` 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:

```
> if (!requireNamespace("BiocManager", quietly=TRUE))
+   install.packages("BiocManager")
> BiocManager::install("topGO")
> BiocManager::install("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	source_5	rank_product	rank_final
627	103	10.0	1.0	NA	1	1.416281	1
79741	NA	NA	8.0	2	NA	2.000000	2
6095	5	2.5	73.5	NA	5	2.058173	3
348980	NA	2.5	20.0	NA	NA	3.535534	4
51365	NA	53.0	3.0	12	27	3.766392	5
7022	88	17.5	5.0	149	3	4.058725	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	source_5	rank_product	rank_final
627	103	10	1.0	NA	1	1	2.0
8897	1	183	282.0	NA	NA	1	2.0
79042	NA	107	99.5	1	NA	1	2.0
7182	2	NA	NA	NA	106	2	5.5
10739	NA	42	2.0	NA	NA	2	5.5
79741	NA	NA	8.0	2	NA	2	5.5

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

457 GO terms scored: 4 terms with p < 0.01

Annotation data:

Annotated genes: 350

Significant genes: 350

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

Nontrivial nodes: 457

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	G0:0065007	biological regulation	0.0017
2	G0:0050789	regulation of biological process	0.0026
3	G0:0050794	regulation of cellular process	0.0045
4	G0:0044087	regulation of cellular component biogene...	0.0062
5	G0:0042692	muscle cell differentiation	0.0121
6	G0:0043085	positive regulation of catalytic activit...	0.0134
7	G0:0010468	regulation of gene expression	0.0137
8	G0:0044089	positive regulation of cellular componen...	0.0141
9	G0:0060255	regulation of macromolecule metabolic pr...	0.0145
10	G0:0010556	regulation of macromolecule biosynthetic...	0.0160
11	G0:0006397	mRNA processing	0.0166
12	G0:0043412	macromolecule modification	0.0198
13	G0:0036211	protein modification process	0.0209
14	G0:0043254	regulation of protein-containing complex...	0.0243
15	G0:0050790	regulation of catalytic activity	0.0245
16	G0:0051146	striated muscle cell differentiation	0.0256
17	G0:0019222	regulation of metabolic process	0.0291
18	G0:0051252	regulation of RNA metabolic process	0.0294
19	G0:0006351	DNA-templated transcription	0.0321
20	G0:0065009	regulation of molecular function	0.0361

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 4.6.0 (2026-04-24), x86_64-pc-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_US.UTF-8, LC_COLLATE=en_US.UTF-8, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C
- Time zone: Etc/UTC
- TZcode source: system (glibc)
- Running under: Ubuntu 24.04.4 LTS
- Matrix products: default
- BLAS: /usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3

- LAPACK:
/usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblas-p0.3.26.so
; LAPACK version3.12.0
- Base packages: base, datasets, graphics, grDevices, methods, stats, stats4, utils
- Other packages: AnnotationDbi 1.74.0, Biobase 2.72.0, BiocGenerics 0.58.1, generics 0.1.4, GO.db 3.23.1, graph 1.90.0, IRanges 2.46.0, miRNAAtap 1.46.0, miRNAAtap.db 0.99.10, org.Hs.eg.db 3.23.1, S4Vectors 0.50.1, SparseM 1.84-2, topGO 2.64.0
- Loaded via a namespace (and not attached): Biostrings 2.80.1, bit 4.6.0, bit64 4.8.2, blob 1.3.0, buildtools 1.0.0, cachem 1.1.0, chron 2.3-62, cli 3.6.6, compiler 4.6.0, crayon 1.5.3, DBI 1.3.0, evaluate 1.0.5, fastmap 1.2.0, glue 1.8.1, grid 4.6.0, gsubfn 0.7, httr 1.4.8, KEGGREST 1.52.2, knitr 1.51, lattice 0.22-9, lifecycle 1.0.5, magrittr 2.0.5, maketools 1.3.2, matrixStats 1.5.0, memoise 2.0.1, otel 0.2.0, pkgconfig 2.0.3, plyr 1.8.9, png 0.1-9, proto 1.0.0, R6 2.6.1, Rcpp 1.1.1-1.1, rlang 1.2.0, RSQLite 3.53.2, Seqinfo 1.2.0, sqldf 0.4-12, stringi 1.8.7, stringr 1.6.0, sys 3.4.3, tools 4.6.0, vctrs 0.7.3, xfun 0.58, XVector 0.52.0

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