The multiMiR user's guide

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1 Introduction

microRNAs (miRNAs) regulate expression by promoting degradation or repressing translation of target transcripts. miRNA target sites have been catalogued in databases based on experimental validation and computational prediction using a variety of algorithms. Several online resources provide collections of multiple databases but need to be imported into other software, such as R, for processing, tabulation, graphing and computation. Currently available miRNA target site packages in R are limited in the number of databases, types of databases and flexibility. The R package *multiMiR*, with web server at `http://multimir.ucdenver.edu`, is a comprehensive collection of predicted and validated miRNA-target interactions and their associations with diseases and drugs. *multiMiR* includes several novel features not available in existing R packages: 1) compilation of 14 different databases, more than any other collection; 2) expansion of databases to those based on disease annotation and drug response, in addition to many experimental and computational databases; and 3) user-defined cutoffs for predicted binding strength to provide the most confident selection. The *multiMiR* package enables retrieval of miRNA-target interactions from 14 external databases in R without the need to visit all these databases. Advanced users can also submit SQL queries to the web server to retrieve results.

2 Getting to know the multiMiR database

The *multiMiR* web server (`http://multimir.ucdenver.edu`) hosts a database containing miRNA-target interactions from external databases. The package *multiMiR* provides functions to communicate with the *multiMiR* web server and its database. Information from each external database is stored in a table in the *multiMiR* database. To see a list of the tables, we can use the `multimir_dbTables` function.

```
> library(multiMiR)
> db.tables = multimir_dbTables()
> db.tables

   Tables_in_multimir
1         diana_microt
2               elmmo
3           map_counts
4         map_metadata
5             metadata
6             microcosm
7           mir2disease
8               miranda
```

```
9            mirdb
10        mirecords
11           mirna
12       mirtarbase
13      pharmaco_mir
14         phenomir
15           pictar
16             pita
17          tarbase
18           target
19        targetscan
```

To display the database schema, we can use the `multimir_dbSchema`
function.

```
> multimir_dbSchema()

--
-- Table structure for table `mirna`
--

DROP TABLE IF EXISTS `mirna`;
CREATE TABLE `mirna` (
  mature_mirna_uid INTEGER UNSIGNED AUTO_INCREMENT,  -- mature miRNA unique ID
  org VARCHAR(4) NOT NULL,                           -- organism abbreviation
  mature_mirna_acc VARCHAR(20) default NULL,         -- mature miRNA accession
  mature_mirna_id VARCHAR(20) default NULL,          -- mature miRNA ID/name
  PRIMARY KEY (mature_mirna_uid),
  KEY org (org),
  KEY mature_mirna_acc (mature_mirna_acc),
  KEY mature_mirna_id (mature_mirna_id)
);

--
-- Table structure for table `target`
--

DROP TABLE IF EXISTS `target`;
CREATE TABLE `target` (
  target_uid INTEGER UNSIGNED AUTO_INCREMENT,   -- target gene unique ID
  org VARCHAR(4) NOT NULL,                       -- organism abbreviation
  target_symbol VARCHAR(80) default NULL,        -- target gene symbol
  target_entrez VARCHAR(10) default NULL,        -- target gene Entrez gene ID
  target_ensembl VARCHAR(20) default NULL,       -- target gene Ensembl gene ID
  PRIMARY KEY (target_uid),
  KEY org (org),
  KEY target_symbol (target_symbol),
  KEY target_entrez (target_entrez),
  KEY target_ensembl (target_ensembl)
);

--
-- Table structure for table `mirecords`
--
```

```
DROP TABLE IF EXISTS `mirecords`;
CREATE TABLE `mirecords` (
  mature_mirna_uid INTEGER UNSIGNED NOT NULL,   -- mature miRNA unique ID
  target_uid INTEGER UNSIGNED NOT NULL,         -- target gene unique ID
  target_site_number INT(10) default NULL,      -- target site number
  target_site_position INT(10) default NULL,    -- target site position
  experiment VARCHAR(160) default NULL,         -- supporting experiment
  support_type VARCHAR(40) default NULL,        -- type of supporting experiment
  pubmed_id VARCHAR(10) default NULL,           -- PubMed ID
  FOREIGN KEY (mature_mirna_uid)
    REFERENCES mirna(mature_mirna_uid)
    ON UPDATE CASCADE ON DELETE RESTRICT,
  FOREIGN KEY (target_uid)
    REFERENCES target(target_uid)
    ON UPDATE CASCADE ON DELETE RESTRICT
);

......

(Please note that only three of the 19 tables are shown here for demonstration purpose.)
```

The function `multimir_dbInfo` will display information of external miRNA and miRNA-target databases in *multiMiR*, including version, release date, link to download the data, and the corresponding table in *multiMiR*.

```
> db.info = multimir_dbInfo()
> db.info

        map_name                      source_name source_version  source_date
1    diana_microt                     DIANA-microT             5   Sept, 2013
2           elmmo                            EIMMo             5    Jan, 2011
3        microcosm                         MicroCosm           5   Sept, 2009
4       mir2disease                      miR2Disease              Mar 14, 2011
5          miranda                          miRanda              Aug, 2010
6            mirdb                            miRDB             4    Jan, 2012
7        mirecords                        miRecords           4 Apr 27, 2013
8       mirtarbase                       miRTarBase         4.5  Nov 1, 2013
9     pharmaco_mir Pharmaco-miR (Verified Sets)
10         phenomir                          PhenomiR           2 Feb 15, 2011
11           pictar                           PicTar           2 Dec 21, 2012
12             pita                             PITA           6 Aug 31, 2008
13          tarbase                          TarBase           6        2012
14        targetscan                      TargetScan         6.2    Jun, 2012
                                                              source_url
1  http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index
2                   http://www.mirz.unibas.ch/miRNAtargetPredictionBulk.php
3       http://www.ebi.ac.uk/enright-srv/microcosm/cgi-bin/targets/v5/download.pl
4                                          http://www.mir2disease.org
5                  http://www.microrna.org/microrna/getDownloads.do
6                                               http://mirdb.org
7                          http://mirecords.biolead.org/download.php
8                   http://mirtarbase.mbc.nctu.edu.tw/php/download.php
9                   http://www.pharmaco-mir.org/home/download_VERSE_db
10                     http://mips.helmholtz-muenchen.de/phenomir/
11                                       http://dorina.mdc-berlin.de
```

```
12                           http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html
13       http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index
14        http://www.targetscan.org/cgi-bin/targetscan/data_download.cgi?db=vert_61
```

Among the 14 external databases, eight (DIANA-microT-CDS, ElMMo, MicroCosm, miRanda, miRDB, PicTar, PITA, and TargetScan) contain predicted miRNA-target interactions. Three of them (miRecords, miRTar-Base, and TarBase) have experimentally validated miRNA-target interactions. The remaining three databases (miR2Disease, Pharmaco-miR, and PhenomiR) contain miRNA-drug/disease associations.

To see how many records are in these 14 external databases we refer to the `multimir_dbCount` function.

```
> db.count = multimir_dbCount()
> db.count

        map_name human_count mouse_count rat_count total_count
1   diana_microt     7664602     3747171         0    11411773
2          elmmo     3959112     1449133    547191     5955436
3       microcosm      762987      534735    353378     1651100
4     mir2disease        2875           0         0        2875
5         miranda     5429955     2379881    247368     8057204
6           mirdb      722269      348778    109409     1180456
7       mirecords        2425         449       171        3045
8       mirtarbase      47003        9448       331       56782
9     pharmaco_mir        308           5         0         313
10       phenomir       15138         491         0       15629
11         pictar      404066      302236         0      706302
12           pita     7710936     5163153         0    12874089
13        tarbase       23447        9024        67       32538
14      targetscan     6298122     2590814         0     8888936

> apply(db.count[,-1], 2, sum)

human_count mouse_count   rat_count total_count
   33043245    16535318     1257915    50836478
```

The current version of *multiMiR* contains nearly 50 million records.

3   List miRNAs, genes, drugs and diseases in the multiMiR database

In addition to functions displaying database and table information, the *multiMiR* package also provides the `list.multimir` function to list all the unique miRNAs, target genes, drugs, and diseases in the *multiMiR* database.

```
> miRNAs = list.multimir("mirna")
> dim(miRNAs)
```

```
[1] 4210     4

> head(miRNAs)

  mature_mirna_uid org mature_mirna_acc mature_mirna_id
1                1 hsa    MIMAT0000078  hsa-miR-23a-3p
2                2 mmu    MIMAT0001422  mmu-miR-434-3p
3                3 mmu    MIMAT0001421  mmu-miR-434-5p
4                4 mmu    MIMAT0000139  mmu-miR-127-3p
5                5 mmu    MIMAT0001420  mmu-miR-433-3p
6                6 mmu    MIMAT0001419  mmu-miR-433-5p

> genes = list.multimir("gene")
> dim(genes)

[1] 94792     5

> head(genes)

  target_uid org target_symbol target_entrez    target_ensembl
1          1 hsa      C21orf33          8209    ENSG00000160221
2          2 mmu          Rtl1        353326 ENSMUSG00000085925
3          3 hsa         SMAD1          4086    ENSG00000170365
4          4 hsa        CXCL12          6387    ENSG00000107562
5          5 hsa        POU4F2          5458    ENSG00000151615
6          6 hsa          MYCN          4613    ENSG00000134323

> drugs = list.multimir("drug")
> dim(drugs)

[1] 64  1

> head(drugs)

                  drug
1 3,3'-diindolylmethane
2         5-fluoroucil
3              abt-737
4          alitretinoin
5       arabinocytosine
6       arsenic trioxide

> diseases = list.multimir("disease")
> dim(diseases)

[1] 223   1

> head(diseases)

                                              disease
1 ACTH-INDEPENDENT MACRONODULAR ADRENAL HYPERPLASIA; AIMAH
2                     ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)
3                     ACUTE MYELOGENEOUS LEUKEMIA (AML)
4                         ACUTE MYELOID LEUKEMIA (AML)
5                     ACUTE PROMYELOCYTIC LEUKEMIA (APL)
6                                              ADENOMA
```

The current version of *multiMiR* has 4210 miRNAs and 94792 target genes from human, mouse, and rat, as well as 64 drugs and 223 disease terms. Depending on the speed of your Internet connection, it may take a few minutes to retrieve the large number of target genes.

4   Use `get.multimir` to query the multiMiR database

`get.multimir` is the main function in the package to retrieve predicted and validated miRNA-target interactions and their disease and drug associations from the *multiMiR* database.

To get familiar with the parameters in `get.multimir`, you can type *?get.multimir* or *help(get.multimir)* in R. In the next section, many examples illustrate the use of the parameters.

5   Examples of multiMiR queries

In this section a variety of examples are described on how to query the multiMiR database.

5.1   Example 1: Retrieve all validated target genes of a given miRNA

In the first example, we ask what genes are validated targets of hsa-miR-18a-3p.

```
> # The default is to search validated interactions in human
> example1 = get.multimir(mirna='hsa-miR-18a-3p', summary=TRUE)

Searching mirecords ...
Searching mirtarbase ...
Searching tarbase ...

> names(example1)

[1] "validated" "summary"

> # Detailed information of the validated miRNA-target interaction
> head(example1$validated)

    database mature_mirna_acc mature_mirna_id target_symbol target_entrez  target_ensembl
1  mirecords     MIMAT0002891  hsa-miR-18a-3p          KRAS          3845 ENSG00000133703
2 mirtarbase     MIMAT0002891  hsa-miR-18a-3p          ACLY            47 ENSG00000260245
3 mirtarbase     MIMAT0002891  hsa-miR-18a-3p          ACLY            47 ENSG00000131473
4 mirtarbase     MIMAT0002891  hsa-miR-18a-3p         AP1B1           162 ENSG00000100280
5 mirtarbase     MIMAT0002891  hsa-miR-18a-3p          ARF5           381 ENSG00000004059
```

```
6 mirtarbase     MIMAT0002891 hsa-miR-18a-3p           ATP5A1           498 ENSG00000152234
                                   experiment          support_type pubmed_id
1 Western blot//Luciferase activity assay                              19372139
2                                CLASH Functional MTI (Weak) 23622248
3                                CLASH Functional MTI (Weak) 23622248
4                                CLASH Functional MTI (Weak) 23622248
5                                CLASH Functional MTI (Weak) 23622248
6                                CLASH Functional MTI (Weak) 23622248

> # Which interactions are supported by Luciferase assay?
> example1$validated[grep("Luciferase", example1$validated[,"experiment"]),]

    database mature_mirna_acc mature_mirna_id target_symbol target_entrez  target_ensembl
1   mirecords     MIMAT0002891 hsa-miR-18a-3p          KRAS         3845 ENSG00000133703
48 mirtarbase     MIMAT0002891 hsa-miR-18a-3p          KRAS         3845 ENSG00000133703
                                     experiment    support_type pubmed_id
1           Western blot//Luciferase activity assay               19372139
48 Luciferase reporter assay//qRT-PCR//Western blot Functional MTI  19372139

> example1$summary[example1$summary[,"target_symbol"] == "KRAS",]

  mature_mirna_acc mature_mirna_id target_symbol target_entrez  target_ensembl mirecords mirtarbase
1     MIMAT0002891 hsa-miR-18a-3p          KRAS         3845 ENSG00000133703         1          1
  validated.sum all.sum
1             2       2
```

It turns out that *KRAS* is the only target validated by Luciferase assay. The interaction was recorded in miRecords and miRTarBase and supported by the same literature, whose PubMed ID is in column 'pubmed_id'. The summary (by setting *"summary=TRUE"* when calling `get.multimir`) shows the number of records in each of the external databases and the total number of databases supporting the interaction.

5.2 Example 2: Retrieve miRNA-target interactions associated with a given drug or disease

In this example we would like to know which miRNAs and their target genes are associated with Cisplatin, a chemotherapy drug used in several cancers.

```
> example2 = get.multimir(disease.drug='cisplatin', table='disease.drug')

Searching mir2disease ...
Searching pharmaco_mir ...
Searching phenomir ...

> names(example2)

[1] "disease.drug"

> nrow(example2$disease.drug)
```

```
[1] 53

> head(example2$disease.drug)

     database mature_mirna_acc mature_mirna_id target_symbol target_entrez  target_ensembl
1 pharmaco_mir    MIMAT0000772  hsa-miR-345-5p         ABCC1          4363 ENSG00000103222
2 pharmaco_mir    MIMAT0000720 hsa-miR-376c-3p          ALK7
3 pharmaco_mir    MIMAT0000423 hsa-miR-125b-5p          BAK1           578 ENSG00000030110
4 pharmaco_mir                      hsa-miR-34          BCL2           596 ENSG00000171791
5 pharmaco_mir    MIMAT0000318 hsa-miR-200b-3p          BCL2           596 ENSG00000171791
6 pharmaco_mir    MIMAT0000617 hsa-miR-200c-3p          BCL2           596 ENSG00000171791
  disease_drug paper_pubmedID
1    cisplatin       20099276
2    cisplatin       21224400
3    cisplatin       21823019
4    cisplatin       18803879
5    cisplatin       21993663
6    cisplatin       21993663
```

get.multimir returns 53 miRNA-target pairs. For more information, we can always refer to the published papers with PubMed IDs in column 'paper_pubmedID'.

5.3 Example 3: Select miRNAs predicted to target a gene

get.multimir also takes target gene(s) as input. In this example we retrieve miRNAs predicted to target *Gnb1* in mouse. For predicted interactions, the default is to query the top 20% predictions within each external database, which is equivalent to setting parameters *"predicted.cutoff = 20"* and *"predicted.cutoff.type = 'p'"* (for percentage cutoff). Here we search the top 35% among all conserved and nonconserved target sites.

```
> example3 = get.multimir(org="mmu", target="Gnb1", table="predicted", summary=TRUE,
+ predicted.cutoff=35, predicted.cutoff.type="p", predicted.site="all")

Searching diana_microt ...
Searching elmmo ...
Searching microcosm ...
Searching miranda ...
Searching mirdb ...
Searching pictar ...
Searching pita ...
Searching targetscan ...

> names(example3)

[1] "predicted" "summary"

> head(example3$predicted)
```

```
        database mature_mirna_acc   mature_mirna_id target_symbol target_entrez   target_ensembl
1 diana_microt     MIMAT0000663     mmu-miR-218-5p          Gnb1          14688 ENSMUSG00000029064
2 diana_microt     MIMAT0017276     mmu-miR-493-5p          Gnb1          14688 ENSMUSG00000029064
3 diana_microt     MIMAT0000656     mmu-miR-139-5p          Gnb1          14688 ENSMUSG00000029064
4 diana_microt     MIMAT0014946 mmu-miR-3074-2-3p          Gnb1          14688 ENSMUSG00000029064
5 diana_microt     MIMAT0000144     mmu-miR-132-3p          Gnb1          14688 ENSMUSG00000029064
6 diana_microt     MIMAT0020608       mmu-miR-5101          Gnb1          14688 ENSMUSG00000029064
  score
1 0.975
2 0.964
3  0.96
4 0.921
5  0.92
6 0.918

> head(example3$summary)

  mature_mirna_acc mature_mirna_id target_symbol target_entrez     target_ensembl diana_microt
1     MIMAT0000133 mmu-miR-101a-3p          Gnb1          14688 ENSMUSG00000029064            1
2     MIMAT0000616 mmu-miR-101b-3p          Gnb1          14688 ENSMUSG00000029064            1
3     MIMAT0003476 mmu-miR-669b-5p          Gnb1          14688 ENSMUSG00000029064            1
4     MIMAT0000663  mmu-miR-218-5p          Gnb1          14688 ENSMUSG00000029064            1
5     MIMAT0002106 mmu-miR-465a-5p          Gnb1          14688 ENSMUSG00000029064            1
6     MIMAT0003739  mmu-miR-673-5p          Gnb1          14688 ENSMUSG00000029064            1
  elmmo microcosm miranda mirdb pictar pita targetscan predicted.sum all.sum
1     1         1       1     0      2    0          1             6       6
2     1         1       1     0      2    0          1             6       6
3     0         0       1     1      0    1          1             5       5
4     2         0       0     0      0    2          2             4       4
5     1         0       1     0      0    0          2             4       4
6     0         0       0     0      2    1          1             4       4
```

The records in *example3$predicted* are ordered by scores from best to worst within each external database. Once again, the summary option allows us to examine the number of target sites predicted by each external database and the total number of databases predicting the interaction.

Finally we examine how many predictions each of the databases has.

```
> apply(example3$summary[,6:13], 2, function(x) sum(x>0))

diana_microt        elmmo   microcosm      miranda       mirdb      pictar        pita
         108           53           5           44           1           9         132
  targetscan
          58
```

5.4 Example 4: Select miRNA(s) predicted to target most, if not all, of the genes of interest

You may have a list of genes involved in a common biological process. It is interesting to check whether some, or all, of these genes are targeted by the same miRNA(s). Here we have four genes involved in chronic obstructive

pulmonary disease (COPD) in human and want to know what miRNAs target these genes by searching the top 500,000 predictions in each external database.

```
> example4 = get.multimir(org='hsa', target=c('AKT2','CERS6','S1PR3','SULF2'), table='predicted',
+ summary=TRUE, predicted.cutoff.type='n', predicted.cutoff=500000)

Searching diana_microt ...
Searching elmmo ...
Searching microcosm ...
Searching miranda ...
Searching mirdb ...
Searching pictar ...
Searching pita ...
Searching targetscan ...
```

Then we count the number of target genes for each miRNA.

```
> example4.counts = addmargins(table(example4$summary[,2:3]))
> example4.counts = example4.counts[-nrow(example4.counts),]
> example4.counts = example4.counts[order(example4.counts[,5], decreasing=TRUE),]
> head(example4.counts)
```

```
                target_symbol
mature_mirna_id  AKT2 CERS6 S1PR3 SULF2 Sum
  hsa-miR-4282      1     1     0     1   3
  hsa-miR-429       1     1     0     1   3
  hsa-miR-524-5p    1     1     0     1   3
  hsa-miR-548p      0     1     1     1   3
  hsa-miR-876-5p    0     1     1     1   3
  hsa-let-7a-5p     1     0     0     1   2
```

5.5 Example 5: Retrieve interactions between a set of miR-NAs and a set of genes

In this example, we profiled miRNA and mRNA expression in poorly metastatic bladder cancer cell lines T24 and Luc, and their metastatic derivatives FL4 and Lul2, respectively. We identified differentially expressed miRNAs and genes between the metastatic and poorly metastatic cells. Let's load the data.

```
> load(url("http://multimir.ucdenver.edu/bladder.rda"))
```

Variable *DE.miRNA.up* contains 9 up-regulated miRNAs and variable *DE.entrez.dn* has 47 down-regulated genes in the two metastatic cell lines. The hypothesis is that interactions between these miRNAs and genes whose expression changed at opposite directions may play a role in cancer metastasis. So we use *multiMiR* to check whether any of the nine miRNAs could target any of the 47 genes.

```
> # search all tables & top 10% predictions
> example5 = get.multimir(org="hsa", mirna=DE.miRNA.up, target=DE.entrez.dn, table="all",
+ summary=TRUE, predicted.cutoff.type="p", predicted.cutoff=10)

Searching diana_microt ...
Searching elmmo ...
Searching microcosm ...
Searching miranda ...
Searching mirdb ...
Searching pictar ...
Searching pita ...
Searching targetscan ...
Searching mirecords ...
Searching mirtarbase ...
Searching tarbase ...
Searching mir2disease ...
Searching pharmaco_mir ...
Searching phenomir ...
```

In the result, there are 3 unique miRNA-gene pairs that have been validated.

```
> example5$validated
```

	database	mature_mirna_acc	mature_mirna_id	target_symbol	target_entrez
1	mirtarbase	MIMAT0000087	hsa-miR-30a-5p	FDX1	2230
2	mirtarbase	MIMAT0000087	hsa-miR-30a-5p	LIMCH1	22998
3	tarbase	MIMAT0000087	hsa-miR-30a-5p	FDX1	2230
4	tarbase	MIMAT0000424	hsa-miR-128	NEK2	4751
5	tarbase	MIMAT0000087	hsa-miR-30a-5p	LIMCH1	22998

	target_ensembl	experiment	support_type	pubmed_id
1	ENSG00000137714	Proteomics	Functional MTI (Weak)	18668040
2	ENSG00000064042	pSILAC//Proteomics;Other	Functional MTI (Weak)	18668040
3	ENSG00000137714	Proteomics	positive	
4	ENSG00000117650	Microarray	positive	
5	ENSG00000064042	Proteomics	positive	

Two miRNAs are associated with bladder cancer in miR2Disease and PhenomiR.

```
> example5$disease.drug[grep("bladder", example5$disease.drug$disease_drug, ignore.case=TRUE),]
```

	database	mature_mirna_acc	mature_mirna_id	target_symbol	target_entrez
18	mir2disease	MIMAT0000418	hsa-miR-23b-3p	NA	NA
711	phenomir	MIMAT0000418	hsa-miR-23b-3p	NA	NA
311	phenomir	MIMAT0000449	hsa-miR-146a-5p	NA	NA

	target_ensembl	disease_drug
18	NA	bladder cancer
711	NA	Bladder cancer
311	NA	Bladder cancer

	paper_pubmedID
18	2007. Micro-RNA profiling in kidney and bladder cancers.
711	17826655
311	19127597

The predicted databases predict 65 miRNA-gene pairs between the 9 miRNAs and 28 of the 47 genes.

```
> length(unique(example5$predicted$mature_mirna_id))

[1] 9

> length(unique(example5$predicted$target_entrez))

[1] 28

> unique.pairs = unique(data.frame(miRNA.ID=as.character(example5$predicted$mature_mirna_id),
+ target.Entrez=as.character(example5$predicted$target_entrez)))
> nrow(unique.pairs)

[1] 65

> head(unique.pairs)

        miRNA.ID target.Entrez
1 hsa-miR-182-5p          2017
2 hsa-miR-182-5p          1112
3 hsa-miR-30d-5p         22998
4 hsa-miR-30a-5p         22998
5 hsa-miR-30b-5p         22998
6 hsa-miR-182-5p          5962
```

Results from each of the predicted databases are already ordered by their scores from best to worst.

```
> example5.split = split(example5$predicted, example5$predicted$database)
```

6 Direct query to the database on the multiMiR web server

As shown previously, `get.multimir` is the main function to retrieve information from the *multiMiR* database, which is hosted at http://multimir.ucdenver.edu. The function builds one SQL query for every external database that the user is going to search, submits the query to the web server, and parses, combines, and summarizes results from the web server. For advanced users, there are a couple ways to query the *multiMiR* database without using the *multiMiR* package; but they have to be familiar with SQL queries. In general, users are still advised to use the `get.multimir` function when querying multiple external databases in *multiMiR*.

6.1 Direct query on the web server

The *multiMiR* package communicates with the *multiMiR* database via the script http://multimir.ucdenver.edu/cgi-bin/multimir.pl on the web server. To show all tables in the database, the following URL can be typed in the address bar of a web browser.

> *http://multimir.ucdenver.edu/cgi-bin/multimir.pl?query=show tables*

The content displayed on the resulting web page is similar with what is returned by typing *multimir_dbTables()* in R. Once again, data from each of the external databases is stored in a table in *multiMiR*. There are also tables for miRNAs (table *mirna*) and target genes (table *target*).

To learn about the structure of a table (e.g. DIANA-microT data in table *diana_microt*), users can use URL

> *http://multimir.ucdenver.edu/cgi-bin/multimir.pl?query=describe diana_microt*

Similar with Example 1, the following URL searches for validated target genes of hsa-miR-18a-3p in miRecords.

> *http://multimir.ucdenver.edu/cgi-bin/multimir.pl?query=SELECT m.mature_mirna_acc, m.mature_mirna_id, t.target_symbol, t.target_entrez, t.target_ensembl, i.experiment, i.support_type, i.pubmed_id FROM mirna AS m INNER JOIN mirecords AS i INNER JOIN target AS t ON (m.mature_mirna_uid=i.mature_mirna_uid and i.target_uid=t.target_uid) WHERE m.mature_mirna_id='hsa-miR-18a-3p'*

As you can see, the query is long and searches just one of the three validated tables in *multiMiR*. While in Example 1, one line of R command using the `get.multimir` function searches, combines and summarizes results from all three validated external databases (miRecords, miRTarBase and TarBase).

6.2 Direct query in R

The same direct queries we did above on the web server can be done in R as well. To show all tables in the database:

```
> direct1 = search.multimir(query="show tables")
```

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These are equivalent to using the `multimir_dbTables` function as showed previously.

To show the structure of table *diana_microt*:

```
> direct2 = search.multimir(query="describe diana_microt")
> direct2

          Field           Type Null Key Default Extra
1 mature_mirna_uid int(10) unsigned   NO MUL
2      target_uid int(10) unsigned   NO MUL
3       miTG_score           double   NO MUL
4        UTR3_hit int(10) unsigned   NO
5         CDS_hit int(10) unsigned   NO
```

To search for validated target genes of hsa-miR-18a-3p in miRecords:

```
> direct3 = search.multimir(query="select m.mature_mirna_acc, m.mature_mirna_id, t.target_symbol, t.target_entrez, t
> direct3

  mature_mirna_acc mature_mirna_id target_symbol target_entrez  target_ensembl
1     MIMAT0002891  hsa-miR-18a-3p          KRAS          3845 ENSG00000133703
                              experiment support_type pubmed_id
1 Western blot//Luciferase activity assay              19372139
```

7 Session Info

```
> sessionInfo()

R version 3.1.2 (2014-10-31)
Platform: x86_64-pc-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C               LC_TIME=en_US.UTF-8
 [4] LC_COLLATE=C               LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C                  LC_ADDRESS=C
[10] LC_TELEPHONE=C             LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
[1] multiMiR_2.0

loaded via a namespace (and not attached):
[1] RCurl_1.95-4.5 XML_3.98-1.1   bitops_1.0-6   tools_3.1.2

> warnings()

NULL
```