

Supplementary Materials And Methods

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1. Functions defined for ceRNA models and workflow of method

We defined the functions that can be used with R programming. Briefly, these functions process a given miRNA:gene dataset and convert to graph object. All values that are significant in miRNA:target interactions are stored in edge variables and processed with formulations that are given in previous section. The functions and steps of approach are explained as following (Figure S1 in Supplementary Figures) :

Conversion of dataset: `priming_graph()` function processes the given dataset that includes competing elements in first variable and repressive element in second variable. If the affinity and/or degradation factors are specified in the function, factors are taken into account, are processed with defaults in vice versa. The formulations that are given in equations (1-4) are performed in this function. This step gives the graph object which contains efficiency values of miRNA:competing target pairs in steady-state in terms of amount. It is assumed that the initial target amounts in the dataset is observed after the repressive activity of miRNAs in steady-state.

Transition of variables in graph: In the previous step, the calculations are performed in the edge variables of the graph object. However, the graph object allows to use node variables, while the node features are handled to the graph. In this direction, `update_nodes` function carries the amount values to node variables. This step must be applied with “once” option because it is primary process.

Trigger change in graph: The dataset are assumed as steady-state in previous step and the efficiency coefficients are calculated according to this acceptance. In the network that is found in steady-state conditions, the change is applied to the graph object for distribution of steady-state. To provide the distribution in the network the workflow offer two methods: `update_variables` and `update_how`. The first, a new dataset that is contained competing and repressive element names and current values of these can be processed with `update_variables`. The second option, the amount of the given node name in `update_how` function can be changed according to “how” argument.

Updating current values of variables: After variables updating in edge variables, these are carried to node variables. Current and previous values of variables are stored as node variables with `update_variables` function.

Simulation of competing behavior of targets: After the change in the steady-state conditions, the network elements try to gain steady-state again. This process progresses as repeating of regulations after the spreading the changes in the network. In this step, simulation of regulations according to given cycle count in `simulate` function is applied. After each simulation cycle, the miRNA repression values are re-calculated and the current values of competing elements are found and saved. The process is performed in the edge data and at the same time outputs of the calculations are carried from edge to node data.

The node elements in the dataset are handled as two type; repressive (miRNAs) and competing (targets). It is assumed in approach that while targets are degrading or inhibiting by miRNAs continuously, miRNAs reversibly used. If the trigger of the network is a miRNA, it maintains the current value of amount that provides by user. On the contrary, it tries to help this process to provide steady-state through the regulations on its amount, if a competing element is used as a trigger. The functions that are used in the approach are developed with R programming so as can be used with other packages. These are can be found in the Github repository [ceRNAetsim Github page](#) and improved with contributions from others.

```
# install.packages('devtools')
# devtools::install_github('selcenari/ceRNAetsim')
library(ceRNAetsim)
```

- load *minsamp* data

```
minsamp <- readRDS("data/minsamp.RDS")
```

```
minsamp
```

```
##   competing miRNA Competing_expression miRNA_expression seed_type region
## 1   Gene1  Mir1           10000           1000      0.43  0.30
## 2   Gene2  Mir1           10000           1000      0.43  0.01
## 3   Gene3  Mir1            5000           1000      0.32  0.40
## 4   Gene4  Mir1           10000           1000      0.23  0.50
## 5   Gene4  Mir2           10000           2000      0.35  0.90
## 6   Gene5  Mir2            5000           2000      0.05  0.40
## 7   Gene6  Mir2           10000           2000      0.01  0.80
##   energy
## 1    -20
## 2    -15
## 3    -14
## 4    -10
## 5    -12
## 6    -11
## 7    -25
```

See Table S1 in Supplementary Tables file.

***minsamp* dataset analysis in lack of interaction factors.**

Firstly, we have analysed minimal data without interaction factors between miRNA:target.

1. We have evaluated graph in the steady state conditions as followings (Figure S2 in Supplementary Figures):

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression)
```

```
## # A tbl_graph: 8 nodes and 7 edges
## #
## # A rooted tree
## #
## # Node Data: 8 x 7 (active)
##   name  type  node_id initial_count count_pre count_current
##   <chr> <chr>   <int>      <dbl>    <dbl>    <dbl>
## 1 Gene1 Comp~     1        10000    10000    10000
## 2 Gene2 Comp~     2        10000    10000    10000
## 3 Gene3 Comp~     3         5000     5000     5000
## 4 Gene4 Comp~     4        10000    10000    10000
```

```
## 5 Gene5 Comp~      5      5000      5000      5000
## 6 Gene6 Comp~      6     10000     10000     10000
## # ... with 2 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 7 x 19
##   from      to Competing_name miRNA_name Competing_expre~ miRNA_expression
##   <int> <int> <chr>           <chr>           <dbl>           <dbl>
## 1     1     7 Gene1           Mir1           10000           1000
## 2     2     7 Gene2           Mir1           10000           1000
## 3     3     7 Gene3           Mir1           5000            1000
## # ... with 4 more rows, and 13 more variables: dummy <dbl>,
## #   aff_factor <dbl>, degg_factor <dbl>, comp_count_list <list>,
## #   comp_count_pre <dbl>, comp_count_current <dbl>,
## #   mirna_count_list <list>, mirna_count_pre <dbl>,
## #   mirna_count_current <dbl>, mirna_count_per_dep <dbl>,
## #   effect_current <dbl>, effect_pre <dbl>, effect_list <list>
```

- 2. We have obtained graph after change on Gene2 expression as following (Figure S3 in Supplementary Figures):

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression) %>% update_how("Gene2",
  2)
```

```
## # A tbl_graph: 8 nodes and 7 edges
## #
## # A rooted tree
## #
## # Node Data: 8 x 7 (active)
##   name type node_id initial_count count_pre count_current
##   <chr> <chr>   <int>         <dbl>    <dbl>    <dbl>
## 1 Gene1 Comp~     1         10000    10000    10000
## 2 Gene2 Comp~     2         10000    10000    20000
## 3 Gene3 Comp~     3          5000     5000     5000
## 4 Gene4 Comp~     4         10000    10000    10000
## 5 Gene5 Comp~     5          5000     5000     5000
## 6 Gene6 Comp~     6         10000    10000    10000
## # ... with 2 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 7 x 19
##   from      to Competing_name miRNA_name Competing_expre~ miRNA_expression
##   <int> <int> <chr>           <chr>           <dbl>           <dbl>
## 1     1     7 Gene1           Mir1           10000           1000
## 2     2     7 Gene2           Mir1           10000           1000
## 3     3     7 Gene3           Mir1           5000            1000
## # ... with 4 more rows, and 13 more variables: dummy <dbl>,
## #   aff_factor <dbl>, degg_factor <dbl>, comp_count_list <list>,
## #   comp_count_pre <dbl>, comp_count_current <dbl>,
## #   mirna_count_list <list>, mirna_count_pre <dbl>,
## #   mirna_count_current <dbl>, mirna_count_per_dep <dbl>,
## #   effect_current <dbl>, effect_pre <dbl>, effect_list <list>
```

- 3. We have determined regulations after Gene2 upregulation (Figure S4 in Supplementary Figures):

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression) %>% update_how("Gene2",
  2) %>% simulate(cycle = 2)
```

```
## # A tbl_graph: 8 nodes and 7 edges
## #
## # A rooted tree
## #
## # Node Data: 8 x 7 (active)
##   name type node_id initial_count count_pre count_current
##   <chr> <chr>   <int>         <dbl>    <dbl>      <dbl>
## 1 Gene1 Comp~    1          10000    10063.    10062.
## 2 Gene2 Comp~    2          10000    19841.    19845.
## 3 Gene3 Comp~    3           5000     5032.     5031.
## 4 Gene4 Comp~    4          10000    10063.    10059.
## 5 Gene5 Comp~    5           5000     5000.     5001.
## 6 Gene6 Comp~    6          10000    10000.    10002.
## # ... with 2 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 7 x 20
##   from to Competing_name miRNA_name Competing_expre~ miRNA_expression
##   <int> <int> <chr>         <chr>         <dbl>          <dbl>
## 1 1 7 Gene1      Mir1      10000          1000
## 2 2 7 Gene2      Mir1      10000          1000
## 3 3 7 Gene3      Mir1       5000           1000
## # ... with 4 more rows, and 14 more variables: dummy <dbl>,
## #   afff_factor <dbl>, degg_factor <dbl>, comp_count_list <list>,
## #   comp_count_pre <dbl>, comp_count_current <dbl>,
## #   mirna_count_list <list>, mirna_count_pre <dbl>,
## #   mirna_count_current <dbl>, mirna_count_per_dep <dbl>,
## #   effect_current <dbl>, effect_pre <dbl>, effect_list <list>,
## #   mirna_count_per_comp <dbl>
```

Note that the regulations are colored according to expression changes of present and a previous value. So, it can be observed that whole gene expressions increase in comparison of initial steady-state. The overall regulations of gene expressions are as followings:

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression) %>% update_how("Gene2",
  2) %>% simulate(2) %>% activate(edges) %>% as_tibble() %>%
  select(Competing_name, comp_count_list, effect_list) %>%
  unnest()
```

```
## # A tibble: 21 x 3
##   Competing_name comp_count_list effect_list
##   <chr>          <dbl>          <dbl>
## 1 Gene1          10000          286.
## 2 Gene1          10063.          222.
## 3 Gene1          10062.          224.
## 4 Gene2          10000          286.
## 5 Gene2          19841.          444.
## 6 Gene2          19845.          441.
```

```
## 7 Gene3          5000          143.
## 8 Gene3          5032.         111.
## 9 Gene3          5031.         112.
## 10 Gene4         10000         286.
## # ... with 11 more rows
```

minsamp dataset analysis with interaction factors.

We have made the same analysis in presence of interaction factors (Sequentially shown at Figure S5-7 in Supplementary Figures).

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region)
```

```
## # A tbl_graph: 8 nodes and 7 edges
## #
## # A rooted tree
## #
## # Node Data: 8 x 7 (active)
##   name type node_id initial_count count_pre count_current
##   <chr> <chr>   <int>      <dbl>    <dbl>    <dbl>
## 1 Gene1 Comp~    1        10000    10000    10000
## 2 Gene2 Comp~    2        10000    10000    10000
## 3 Gene3 Comp~    3         5000     5000     5000
## 4 Gene4 Comp~    4        10000    10000    10000
## 5 Gene5 Comp~    5         5000     5000     5000
## 6 Gene6 Comp~    6        10000    10000    10000
## # ... with 2 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 7 x 22
##   from to Competing_name miRNA_name Competing_expre~ miRNA_expression
##   <int> <int> <chr>          <chr>          <dbl>          <dbl>
## 1 1 7 Gene1      Mir1      10000          1000
## 2 2 7 Gene2      Mir1      10000          1000
## 3 3 7 Gene3      Mir1      5000           1000
## # ... with 4 more rows, and 16 more variables: energy <dbl>,
## # seed_type <dbl>, region <dbl>, dummy <dbl>, aff_factor <dbl>,
## # degg_factor <dbl>, comp_count_list <list>, comp_count_pre <dbl>,
## # comp_count_current <dbl>, mirna_count_list <list>,
## # mirna_count_pre <dbl>, mirna_count_current <dbl>,
## # mirna_count_per_dep <dbl>, effect_current <dbl>, effect_pre <dbl>,
## # effect_list <list>
```

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% update_how("Gene2",
  2)
```

```
## # A tbl_graph: 8 nodes and 7 edges
## #
## # A rooted tree
```

```
## #
## # Node Data: 8 x 7 (active)
##   name type node_id initial_count count_pre count_current
##   <chr> <chr>   <int>         <dbl>    <dbl>      <dbl>
## 1 Gene1 Comp~    1           10000    10000      10000
## 2 Gene2 Comp~    2           10000    10000      20000
## 3 Gene3 Comp~    3            5000     5000       5000
## 4 Gene4 Comp~    4           10000    10000      10000
## 5 Gene5 Comp~    5            5000     5000       5000
## 6 Gene6 Comp~    6           10000    10000      10000
## # ... with 2 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 7 x 22
##   from to Competing_name miRNA_name Competing_expre~ miRNA_expression
##   <int> <int> <chr>         <chr>         <dbl>          <dbl>
## 1     1     7 Gene1      Mir1           10000          1000
## 2     2     7 Gene2      Mir1           10000          1000
## 3     3     7 Gene3      Mir1            5000          1000
## # ... with 4 more rows, and 16 more variables: energy <dbl>,
## #   seed_type <dbl>, region <dbl>, dummy <dbl>, afff_factor <dbl>,
## #   degg_factor <dbl>, comp_count_list <list>, comp_count_pre <dbl>,
## #   comp_count_current <dbl>, mirna_count_list <list>,
## #   mirna_count_pre <dbl>, mirna_count_current <dbl>,
## #   mirna_count_per_dep <dbl>, effect_current <dbl>, effect_pre <dbl>,
## #   effect_list <list>
```

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% update_how("Gene2",
  2) %>% simulate(cycle = 2)
```

```
## # A tbl_graph: 8 nodes and 7 edges
## #
## # A rooted tree
## #
## # Node Data: 8 x 7 (active)
##   name type node_id initial_count count_pre count_current
##   <chr> <chr>   <int>         <dbl>    <dbl>      <dbl>
## 1 Gene1 Comp~    1           10000    10065.      10064.
## 2 Gene2 Comp~    2           10000    19997.      19997.
## 3 Gene3 Comp~    3            5000     5023.       5023.
## 4 Gene4 Comp~    4           10000    10029.      10028.
## 5 Gene5 Comp~    5            5000     5000       5000.
## 6 Gene6 Comp~    6           10000    10000      10000.
## # ... with 2 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 7 x 23
##   from to Competing_name miRNA_name Competing_expre~ miRNA_expression
##   <int> <int> <chr>         <chr>         <dbl>          <dbl>
## 1     1     7 Gene1      Mir1           10000          1000
## 2     2     7 Gene2      Mir1           10000          1000
## 3     3     7 Gene3      Mir1            5000          1000
## # ... with 4 more rows, and 17 more variables: energy <dbl>,
## #   seed_type <dbl>, region <dbl>, dummy <dbl>, afff_factor <dbl>,
```

```
## #   degg_factor <dbl>, comp_count_list <list>, comp_count_pre <dbl>,
## #   comp_count_current <dbl>, mirna_count_list <list>,
## #   mirna_count_pre <dbl>, mirna_count_current <dbl>,
## #   mirna_count_per_dep <dbl>, effect_current <dbl>, effect_pre <dbl>,
## #   effect_list <list>, mirna_count_per_comp <dbl>
```

When the graphs which were resulted from analyses were examined, it was observed that behaviors were same. But, when the results were analysed in terms of expression values, the regulation differences can be observed.

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% update_how("Gene2",
  2) %>% simulate(3) %>% activate(edges) %>% as_tibble() %>%
  select(Competing_name, comp_count_list, effect_list) %>%
  unnest()
```

```
## # A tibble: 28 x 3
##   Competing_name comp_count_list effect_list
##   <chr>           <dbl>           <dbl>
## 1 Gene1           10000           263.
## 2 Gene1           10065.           198.
## 3 Gene1           10064.           199.
## 4 Gene1           10064.           199.
## 5 Gene2           10000             6.58
## 6 Gene2           19997.            9.91
## 7 Gene2           19997.            9.88
## 8 Gene2           19997.            9.88
## 9 Gene3            5000           91.5
## 10 Gene3          5023.           68.8
## # ... with 18 more rows
```

Common target perturbation in *minsamp* dataset.

Genes targeted by multiple miRNAs (referred to as “common target”) are of special interest since they are subject to cooperative effect. Also, they perturb more than one neighborhood. In our small dataset, minsamp, Gene4 is regulated by two miRNAs. Let’s simulate perturbation effects triggered by Gene4 (Shown at Figure S8 in Supplementary Figures .

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% update_how("Gene4",
  2) %>% simulate(cycle = 2)
```

```
## # A tbl_graph: 8 nodes and 7 edges
## #
## # A rooted tree
## #
## # Node Data: 8 x 7 (active)
##   name type node_id initial_count count_pre count_current
##   <chr> <chr>   <int>      <dbl>      <dbl>      <dbl>
## 1 Gene1 Comp~     1        10000      10028.      10027.
```

```
## 2 Gene2 Comp~      2      10000      10001.      10001.
## 3 Gene3 Comp~      3       5000       5010.       5009.
## 4 Gene4 Comp~      4      10000      19803.      19806.
## 5 Gene5 Comp~      5       5000       5024.       5024.
## 6 Gene6 Comp~      6      10000      10044.      10044.
## # ... with 2 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 7 x 23
##   from      to Competing_name miRNA_name Competing_expre~ miRNA_expression
##   <int> <int> <chr>           <chr>           <dbl>           <dbl>
## 1     1     7 Gene1           Mir1             10000           1000
## 2     2     7 Gene2           Mir1             10000           1000
## 3     3     7 Gene3           Mir1              5000           1000
## # ... with 4 more rows, and 17 more variables: energy <dbl>,
## #   seed_type <dbl>, region <dbl>, dummy <dbl>, aff_factor <dbl>,
## #   degg_factor <dbl>, comp_count_list <list>, comp_count_pre <dbl>,
## #   comp_count_current <dbl>, mirna_count_list <list>,
## #   mirna_count_pre <dbl>, mirna_count_current <dbl>,
## #   mirna_count_per_dep <dbl>, effect_current <dbl>, effect_pre <dbl>,
## #   effect_list <list>, mirna_count_per_comp <dbl>
```

The common target perturbation (increasing to two fold at Gene4 expression in presence of interaction factors) resulted in more prominent efficiency at the same conditions (shown in following).

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% update_how("Gene4",
  2) %>% simulate(3) %>% activate(edges) %>% as_tibble() %>%
  select(Competing_name, comp_count_list, effect_list) %>%
  unnest()
```

```
## # A tibble: 28 x 3
##   Competing_name comp_count_list effect_list
##   <chr>           <dbl>           <dbl>
## 1 Gene1           10000           263.
## 2 Gene1           10028.          236.
## 3 Gene1           10027.          237.
## 4 Gene1           10027.          237.
## 5 Gene2           10000            6.58
## 6 Gene2           10001.           5.89
## 7 Gene2           10001.           5.90
## 8 Gene2           10001.           5.90
## 9 Gene3            5000           91.5
## 10 Gene3          5010.           81.9
## # ... with 18 more rows
```

Determination of perturbation efficiencies of elements in system.

```
sample_graph <- priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region)
```



```
find_node_perturbation(sample_graph, how = 2, cycle = 3,
  limit = 0.1)
```

```
## # A tibble: 8 x 9
##   name type node_id initial_count count_pre count_current
##   <chr> <chr>   <int>         <dbl>     <dbl>         <dbl>
## 1 Gene1 Comp~     1         10000     10000         10000
## 2 Gene2 Comp~     2         10000     10000         10000
## 3 Gene3 Comp~     3          5000      5000          5000
## 4 Gene4 Comp~     4         10000     10000         10000
## 5 Gene5 Comp~     5          5000      5000          5000
## 6 Gene6 Comp~     6         10000     10000         10000
## 7 Mir1  miRNA     7          1000      1000          1000
## 8 Mir2  miRNA     8          2000      2000          2000
## # ... with 3 more variables: changes_variable <chr>,
## #   perturbation_efficiency <dbl>, perturbed_count <dbl>
```

2. Obtaining breast cancer dataset and integration

This section describes how to apply `ceRnAnetsim` package on a breast cancer patient miRNA:target interaction dataset. Before the approach, we obtained three datasets and combined them.

2.1 How to get gene expression counts of TCGA-E9-A1N5 patient.

We have obtained the gene expression values of patient using `TCGAbiolinks` package from Bioconductor. For this process, we have followed the instructions of the package. `TCGAbiolinks` package provides to obtain data for whole number of given barcode(s) at once. But, we preferred to download them separately to show datasets.

- Obtain to gene expression counts of tumor tissue.

```
BCP_tumor <- GDCquery(project = "TCGA-BRCA", data.category = "Transcriptome Profiling",
  data.type = "Gene Expression Quantification", workflow.type = "HTSeq - Counts",
  barcode = "TCGA-E9-A1N5-01A-11R-A14D-07")

GDCdownload(BCP_tumor)
BCPGE_tumor <- GDCprepare(BCP_tumor)

TCGA_E9_A1N5_tumor <- as.data.frame(assay(BCPGE_tumor)) %>%
  mutate(ensembl_gene_id = rownames(.)) %>% dplyr::inner_join(as.data.frame(rowData(BCPGE_tumor)),
    by = "ensembl_gene_id") %>% dplyr::select(ensembl_gene_id,
    external_gene_name, 1)

colnames(TCGA_E9_A1N5_tumor)[3] <- "GE_tumor"

head(TCGA_E9_A1N5_tumor)
```

- Obtain to gene expression counts of normal tissue.

```
BCP_normal <- GDCquery(project = "TCGA-BRCA", data.category = "Transcriptome Profiling",
  data.type = "Gene Expression Quantification", workflow.type = "HTSeq - Counts",
  barcode = "TCGA-E9-A1N5-11A-41R-A14D-07")
GDCdownload(BCP_normal)

BCPGE_normal <- GDCprepare(BCP_normal)

TCGA_E9_A1N5_normal <- as.data.frame(assay(BCPGE_normal)) %>%
  mutate(ensembl_gene_id = rownames(.)) %>% dplyr::inner_join(as.data.frame(rowData(BCPGE_normal)),
  by = "ensembl_gene_id") %>% dplyr::select(ensembl_gene_id,
  external_gene_name, 1)

colnames(TCGA_E9_A1N5_normal)[3] <- "GE_normal"

head(TCGA_E9_A1N5_normal)
```

2.2 How to get miRNA expression counts of TCGA-E9-A1N5 patient.

We have used TCGAbiolinks package to obtain miRNA expression quantification. The query gives read count of miRNA as isoform chromosome coordination. The data also contains mature miRNA information. So, we processed data to attain -5p -3p isoform information using mirbase release21 dataset.

- Get the mirbase id of mature miRNA:

We downloaded the mirbase release 21 dataset from mirbase and processed the patient mirna expression datasets as following:

```
mirbase_id_conv <- read_tsv("hsa_mirna.txt", comment = "#",
  col_names = FALSE) %>% dplyr::select(mirna_type = X3,
  definition = X9) %>% filter(!endsWith(mirna_type,
  "primary_transcript")) %>% tidyr::separate(definition,
  c("ID", "Alias", "Name", "Derivated"), sep = ";") %>%
  dplyr::select(Alias, Name) %>% tidyr::separate(Alias,
  c("trash1", "ID"), sep = "=") %>% tidyr::separate(Name,
  c("trash2", "Name"), sep = "=") %>% dplyr::select(-trash1,
  -trash2)

head(mirbase_id_conv)
```

```
## # A tibble: 6 x 2
##   ID      Name
##   <chr>   <chr>
## 1 MIMAT0027618 hsa-miR-6859-5p
## 2 MIMAT0027619 hsa-miR-6859-3p
## 3 MIMAT0005890 hsa-miR-1302
## 4 MIMAT0027618 hsa-miR-6859-5p
## 5 MIMAT0027619 hsa-miR-6859-3p
## 6 MIMAT0049032 hsa-miR-12136
```

- Obtain the miRNA expression of tumor tissue of patient:

```
BCP_mirnatumor <- GDCquery(project = "TCGA-BRCA", data.category = "Transcriptome Profiling",
  data.type = "Isoform Expression Quantification",
  workflow.type = "BCGSC miRNA Profiling", barcode = "TCGA-E9-A1N5-01A-11R-A14C-13")

GDCdownload(BCP_mirnatumor)

BCPME_mirnatumor <- GDCprepare(BCP_mirnatumor) %>%
  as.data.frame() %>% dplyr::select(miRNA_ID, read_count,
  reads_per_million_miRNA_mapped, miRNA_region) %>%
  dplyr::filter(startsWith(miRNA_region, "mature")) %>%
  dplyr::mutate(mirbase_id = str_remove(miRNA_region,
  "mature,")) %>% dplyr::select(-miRNA_region) %>%
  dplyr::inner_join(mirbase_id_conv, by = c(mirbase_id = "ID")) %>%
  dplyr::select(miRNA_name = Name, read_count, reads_per_million_miRNA_mapped) %>%
  dplyr::group_by(miRNA_name) %>% mutate(read_count = sum(read_count),
  reads_per_million_miRNA_mapped = sum(reads_per_million_miRNA_mapped)) %>%
  dplyr::ungroup() %>% distinct()

head(BCPME_mirnatumor)
```

- Obtain the miRNA expression of normal tissue of patient:

```
BCP_mirnanormal <- GDCquery(project = "TCGA-BRCA",
  data.category = "Transcriptome Profiling", data.type = "Isoform Expression Quantification",
  workflow.type = "BCGSC miRNA Profiling", barcode = "TCGA-E9-A1N5-11A-41R-A14C-13")

GDCdownload(BCP_mirnanormal)
# a616435d-0b69-48ac-813d-5d75ad9b85eb.mirbase21.isoforms.quantification.txt

BCPME_mirnanormal <- GDCprepare(BCP_mirnanormal) %>%
  as.data.frame() %>% dplyr::select(miRNA_ID, read_count,
  reads_per_million_miRNA_mapped, miRNA_region) %>%
  dplyr::filter(startsWith(miRNA_region, "mature")) %>%
  dplyr::mutate(mirbase_id = str_remove(miRNA_region,
  "mature,")) %>% dplyr::select(-miRNA_region) %>%
  dplyr::inner_join(mirbase_id_conv, by = c(mirbase_id = "ID")) %>%
  dplyr::select(miRNA_name = Name, read_count, reads_per_million_miRNA_mapped) %>%
  dplyr::group_by(miRNA_name) %>% mutate(read_count = sum(read_count),
  reads_per_million_miRNA_mapped = sum(reads_per_million_miRNA_mapped)) %>%
  dplyr::ungroup() %>% distinct()

head(BCPME_mirnanormal)
```

2.3 Get the high-throughput experimental miRNA:target dataset.

There are various datasets about miRNA:target pairs such as miRTarBase, DianaTools, miRecords, miRWalk etc. Some of these present the experimentally supported miRNA target pairs or only predicted ones. The experimentally supported datasets generally provides weak evidence for interactions. For these reasons, we obtained the high-throughput experimental miRNA:target dataset from two studies performed by Helwak et al. and Moore et al. These steps were not handle in this file because they contain many processes.

Briefly these datasets contain various common information about miRNA:target interactions such as the miRNA name, miRNAsequence, target name, target sequence, their chromosomal locations, binding location

on the target sequence, binding free energy, seed structure. But these datasets provides the informations with different data structures. So we followed the steps:

- The datasets were directly downloaded from supplementary data files of the studies.
- It was provided that the datasets are converted to same human genome build.
- The seed type information was organized as the same style.
- The datasets were combined.
- We committed the interaction factors as numeric values according to previous studies. (We added the interaction factors and their numeric values at Supplementary files, Process on miRNA:target pairs dataset for process codes and Supplementary Tables for used numeric values)

Finally, we have obtained the experimentally supported miRNA:target dataset.

```
experimentalmirnagene <- readRDS("data/experimentalmirnagene.RDS")

head(experimentalmirnagene)

## # A tibble: 6 x 18
##   cluster chromosome start_position end_position strand hgnc_symbol
##   <chr>    <chr>          <int>         <int> <chr>    <chr>
## 1 0727A-- chr5           162864575     162873157 1       CCNG1
## 2 L1HS-1~ chr14          95552565     95624347 -1      DICER1
## 3 L2HS-8~ chr6          109307640     109416022 -1      SESN1
## 4 L2HS-1~ chr5          36876861     37066515 1       NIPBL
## 5 L2-407~ chr4          106603784     106817143 -1      INTS12
## 6 L1HS-7~ chr5          130977407     131132710 -1      FNIP1
## # ... with 12 more variables: Ensembl_Gene_Id <chr>,
## #   Ensembl_Transcript_Id <chr>, target_seq <chr>, miRNA <chr>,
## #   miR_seq <chr>, seed_type <chr>, Energy <dbl>, HG38build_loc <chr>,
## #   Genom_build <chr>, region <chr>, region_effect <dbl>,
## #   seed_type_effect <dbl>
```

The methods about miRNA:target interactions are based a basic principle that is reading after isolation of miRNA:target chimerics. The datasets contain all the chimeric miRNA:target structures found in the medium during the experiment. On the other hand, it could be said that the reading is performed as snapshot. Because of that, the methods can provide different chimeric interactions the same miRNA:target pair. We have preferred to select most effective interaction parameters for the same miRNA:target pairs that can exhibit various interactions. The step is performed as:

```
tocombine_mirnagene <- experimentalmirnagene %>% dplyr::select(miRNA,
  Ensembl_Gene_Id, hgnc_symbol, Energy, seed_type_effect,
  region_effect) %>% distinct() %>% group_by(Ensembl_Gene_Id,
  miRNA) %>% mutate(seed_type_effect = ifelse(seed_type_effect ==
  max(seed_type_effect), seed_type_effect, max(seed_type_effect)),
  Energy = ifelse(Energy == min(Energy), Energy,
  min(Energy)), region_effect = ifelse(region_effect ==
  max(region_effect), region_effect, max(region_effect))) %>%
  distinct()

head(tocombine_mirnagene)
```

2.4 Combine the dataset

```
E9GE_mirnagenenormal <- BCPME_mirnanormal %>% dplyr::inner_join(tocombine_mirnagene,  
  by = c(miRNA_name = "miRNA")) %>% dplyr::inner_join(TCGA_E9_A1N5_normal,  
  by = c(Ensembl_Gene_Id = "ensembl_gene_id", hgnc_symbol = "external_gene_name")) %>%  
  distinct() %>% dplyr::select(hgnc_symbol, miRNA_name,  
  mirna_RPM = reads_per_million_miRNA_mapped, GE_normal,  
  Energy, seed_type_effect, region_effect)
```

```
E9GE_mirnagenenormal <- readRDS("data/E9GE_mirnagenenormal.RDS")  
head(E9GE_mirnagenenormal)
```

```
## # A tibble: 6 x 7  
##   hgnc_symbol miRNA_name mirna_RPM GE_normal Energy seed_type_effect  
##   <chr>      <chr>      <dbl>   <dbl>  <dbl>      <dbl>  
## 1 CCNG1      hsa-let-7~    111204.    5245  -25.1      0.05  
## 2 DICER1      hsa-let-7~    111204.    3285  -24.4      0.43  
## 3 SESN1      hsa-let-7~    111204.    1179  -22.2      0.05  
## 4 NIPBL      hsa-let-7~    111204.    4503  -22.1      0.05  
## 5 INTS12     hsa-let-7~    111204.     600  -21.9      0.05  
## 6 FNIP1      hsa-let-7~    111204.    1248  -21.8      0.43  
## # ... with 1 more variable: region_effect <dbl>
```

```
E9GE_mirnagenetumor <- BCPME_mirnatumor %>% dplyr::inner_join(tocombine_mirnagene,  
  by = c(miRNA_name = "miRNA")) %>% dplyr::inner_join(TCGA_E9_A1N5_tumor,  
  by = c(Ensembl_Gene_Id = "ensembl_gene_id", hgnc_symbol = "external_gene_name")) %>%  
  distinct() %>% dplyr::select(hgnc_symbol, miRNA_name,  
  mirna_RPM = reads_per_million_miRNA_mapped, GE_tumor,  
  Energy, seed_type_effect, region_effect)
```

```
# saveRDS(E9GE_mirnagenetumor,  
# 'data/E9GE_mirnagenetumor.RDS')
```

```
E9GE_mirnagenetumor <- readRDS("data/E9GE_mirnagenetumor.RDS")  
head(E9GE_mirnagenetumor)
```

```
## # A tibble: 6 x 7  
##   hgnc_symbol miRNA_name mirna_RPM GE_tumor Energy seed_type_effect  
##   <chr>      <chr>      <dbl>   <dbl>  <dbl>      <dbl>  
## 1 CCNG1      hsa-let-7~    62406.    2467  -25.1      0.05  
## 2 DICER1      hsa-let-7~    62406.    5023  -24.4      0.43  
## 3 SESN1      hsa-let-7~    62406.     829  -22.2      0.05  
## 4 NIPBL      hsa-let-7~    62406.    5126  -22.1      0.05  
## 5 INTS12     hsa-let-7~    62406.    1009  -21.9      0.05  
## 6 FNIP1      hsa-let-7~    62406.    2144  -21.8      0.43  
## # ... with 1 more variable: region_effect <dbl>
```

2.5 Selection of trigger node

We have determined the most important nodes of network. We applied `find_node_perturbation` function. We only defined nodes that affect the other nodes more than 1.05 fold change with 10 iteration when they increase 3 fold.

This dataset, perturbationofnetwork, includes totally 423 effective nodes. We selected SERPINE2 gene to perturb the network because it is the most efficient node gene in network.

2.5 Approach of Method into Combined Datasets

We selected SERPINE2 gene for simulation of regulation on network.

2.5.1 Find iteration of simulation

The node amount of changed gene on the system in terms of percentage were shown at Figure S9 in Supplementary Figures. As seen, firstly, the changed gene count increase. The system which contains the hundreds of miRNAs and thousands of genes can slowly gain the steady-state again. At first glance, it can be assumed that when all nodes in the system are reached, stable state will be provided. However, although all nodes are reached, the nodes competing with each other cause the edits to continue for a while.

The dynamics of the approach are shown in package vignettes.

So, we offered an approach about to find iteration. `find_iteration` function does not give the iteration to gain steady-state, but it gives the iteration which has maximum affected node counts. The function is applied as following:

```
as.data.frame(E9GE_mirnagenenormal) %>% priming_graph(competing_count = GE_normal,
  miRNA_count = mirna_RPM, aff_factor = c(Energy,
    seed_type_effect), deg_factor = region_effect) %>%
  update_how("SERPINE2", 2.75) %>% simulate(50) %>%
  find_iteration(limit = 1, plot = FALSE)
```

```
## Warning in priming_graph(., competing_count = GE_normal, miRNA_count = mirna_RPM, : First column is p
```

```
## [1] 31
```

2.5.2 Simulation of dataset

We tried to apply two fold of the point that SERPINE2 has maximum affected genes on network.

```
as.data.frame(E9GE_mirnagenenormal) %>% priming_graph(competing_count = GE_normal,
  miRNA_count = mirna_RPM, aff_factor = c(Energy,
    seed_type_effect), deg_factor = region_effect) %>%
  update_how("SERPINE2", 2.75) %>% simulate(62)
```

```
## Warning in priming_graph(., competing_count = GE_normal, miRNA_count = mirna_RPM, : First column is p
```

```
## # A tbl_graph: 8215 nodes and 25618 edges
```

```
## #
```

```
## # A directed acyclic simple graph with 8 components
```

```
## #
```

```
## # Node Data: 8,215 x 7 (active)
```

```
##   name   type node_id initial_count count_pre count_current
```

```
##   <chr> <chr>   <int>         <dbl>    <dbl>    <dbl>
```

```
## 1 CCNG1 Comp~     1          5245     5249.     5250.
```

```
## 2 DICE~ Comp~     2          3285     3285.     3290.
```

```
## 3 SESN1 Comp~      3      1179      1179.      1179.
## 4 NIPBL Comp~      4      4503      4503.      4504.
## 5 INTS~ Comp~      5        600        600.        600.
## 6 FNIP1 Comp~      6      1248      1247.      1252.
## # ... with 8,209 more rows, and 1 more variable: changes_variable <chr>
## #
## # Edge Data: 25,618 x 23
##   from      to Competing_name miRNA_name GE_normal mirna_RPM Energy
##   <int> <int> <chr>           <chr>         <dbl>    <dbl>  <dbl>
## 1     1    7871 CCNG1         hsa-let-7~    5245    111204. -25.1
## 2     2    7871 DICER1       hsa-let-7~    3285    111204. -24.4
## 3     3    7871 SESN1       hsa-let-7~    1179    111204. -22.2
## # ... with 2.562e+04 more rows, and 16 more variables:
## #   seed_type_effect <dbl>, region_effect <dbl>, dummy <dbl>,
## #   aff_factor <dbl>, degg_factor <dbl>, comp_count_list <list>,
## #   comp_count_pre <dbl>, comp_count_current <dbl>,
## #   mirna_count_list <list>, mirna_count_pre <dbl>,
## #   mirna_count_current <dbl>, mirna_count_per_dep <dbl>,
## #   effect_current <dbl>, effect_pre <dbl>, effect_list <list>,
## #   mirna_count_per_comp <dbl>
```

```
simulation_results <- as.data.frame(E9GE_mirnagenenormal) %>%
  priming_graph(competing_count = GE_normal, miRNA_count = mirna_RPM,
    aff_factor = c(Energy, seed_type_effect), deg_factor = region_effect) %>%
  update_how("SERPINE2", 2.75) %>% simulate(62) %>%
  as_tibble() %>% dplyr::select(name, initial_count,
    count_current)
```

```
## Warning in priming_graph(., competing_count = GE_normal, miRNA_count = mirna_RPM, : First column is
```

2.5.3 Comparison of simulation results and tumor tissue expression values

```
E9GE_mirnagenetumor %>% dplyr::select(hgnc_symbol,
  GE_tumor) %>% dplyr::inner_join((E9GE_mirnagenenormal %>%
  dplyr::select(hgnc_symbol, GE_normal)), by = "hgnc_symbol") %>%
  inner_join(simulation_results, by = c(hgnc_symbol = "name")) %>%
  distinct()
```

```
## # A tibble: 7,812 x 5
##   hgnc_symbol GE_tumor GE_normal initial_count count_current
##   <chr>      <dbl>    <dbl>         <dbl>         <dbl>
## 1 CCNG1      2467      5245          5245          5250.
## 2 DICER1     5023      3285          3285          3290.
## 3 SESN1       829      1179          1179          1179.
## 4 NIPBL     5126      4503          4503          4504.
## 5 INTS12     1009       600           600           600.
## 6 FNIP1      2144      1248          1248          1252.
## 7 ACAD8       860      1249          1249          1249.
## 8 CCNB2       749       690           690           690.
## 9 ZNF260     1808      1067          1067          1067.
## 10 SYVN1      2565      2300          2300          2303.
## # ... with 7,802 more rows
```

Actually, we have developed to provide a new approach mirna mediated regulation networks. This approach may not explain the whole regulation behaviors between miRNAs and targets but can be first step to more detailed and coherent miRNA:target regulation approach.