

Supplementary Figures and Tables

Selcen Ari

Alper Yilmaz

2020-08-19

Contents

1. Supplementary Figures	2
1.1 <i>minsamp</i> dataset analysis in absence of interaction factors.	2
1.2 <i>minsamp</i> dataset analysis with interaction factors.	5
1.3 Common target perturbation in <i>minsamp</i> dataset.	8
2 Supplementary Tables	11
2.1 <i>minsamp</i> dataset	11
2.2 Significant factors in miRNA:target interactions	11
2.3 Content of High-throughput experimental studies	12
2.4 Variables of network object during simulation	13
REFERENCES	14

1. Supplementary Figures

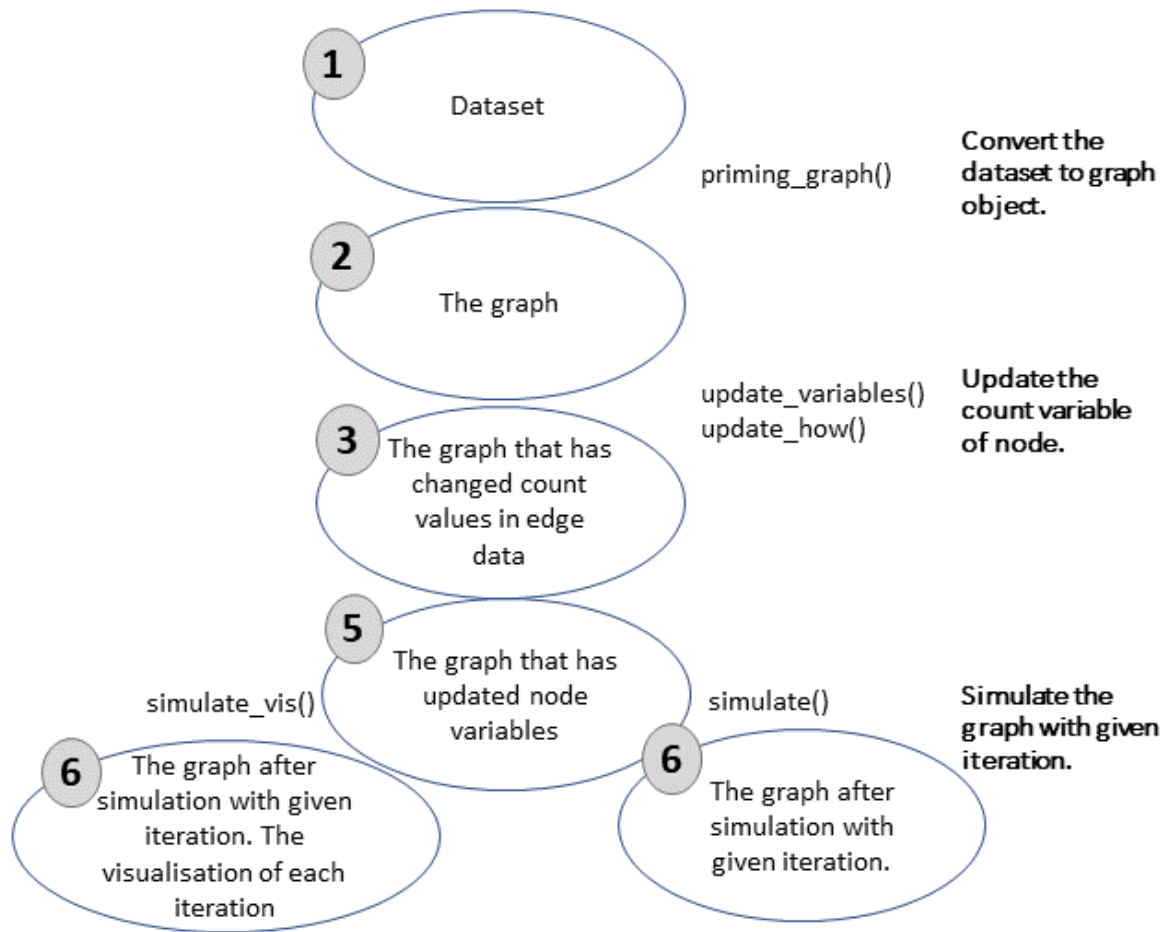


Figure S1: Workflow for simulation of competing endogenous RNA regulations. Graph object in steps 2-6 is saved and updated continuously.

```
library(ceRNAnetsim)
```

1.1 *minsamp* dataset analysis in absence of interaction factors.

```
data("minsamp")
```

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", title = " ")
```

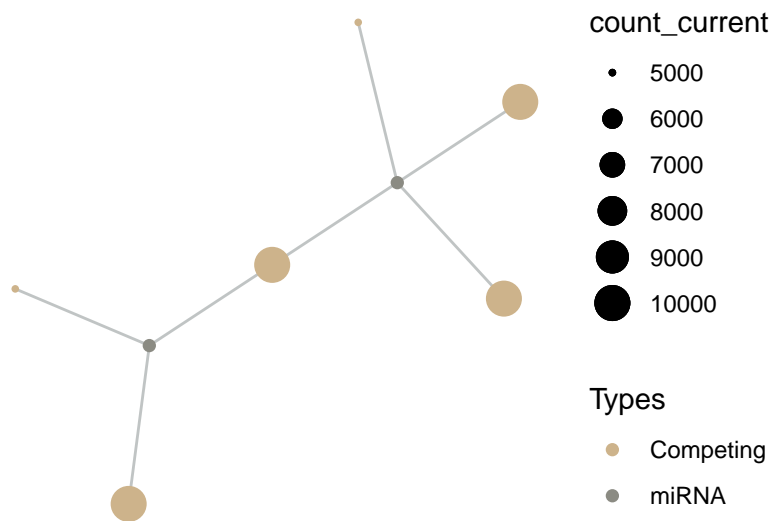


Figure S2: Minimal Dataset in Steady-state

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression) %>% update_how("Gene2",
  2) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", title = " ")
```

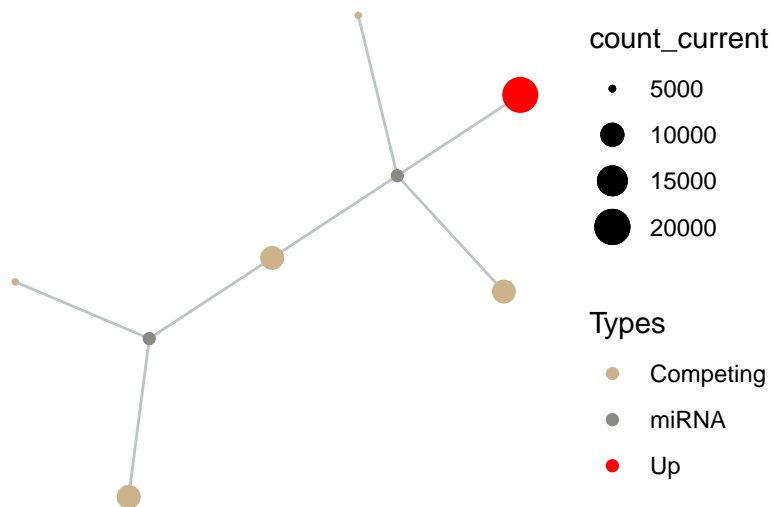


Figure S3: Gene2 Upregulation on Minimal Dataset

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression) %>% update_how("Gene2",
  2) %>% simulate(cycle = 1) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", Downregulation = "blue",
  title = "A")

priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression) %>% update_how("Gene2",
  2) %>% simulate(2) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", Downregulation = "blue",
  title = "B")
```

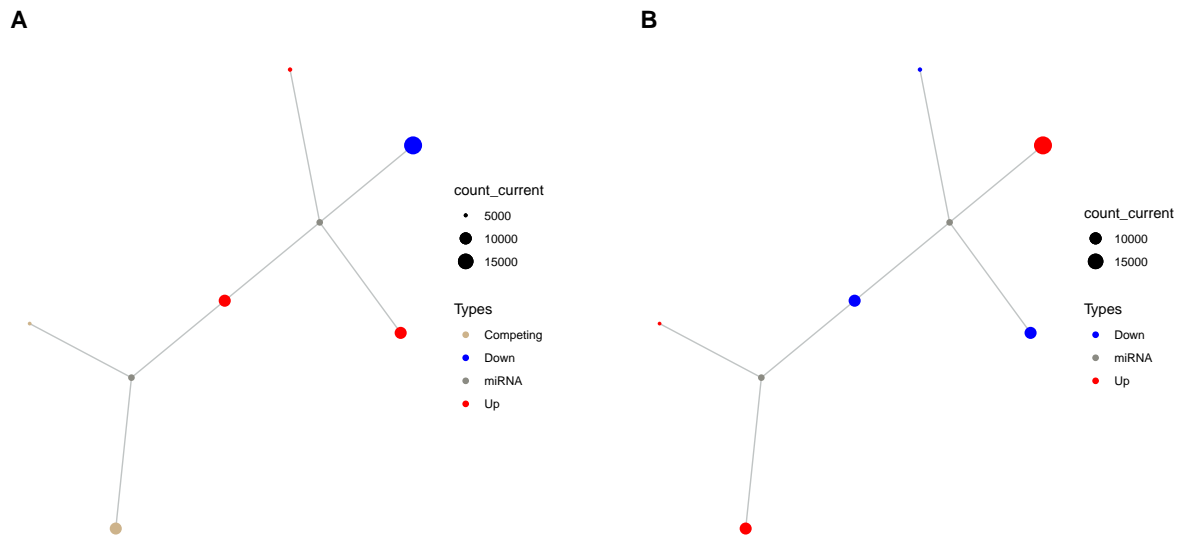


Figure S4: Sequential iteration of minsamp data. A) First response of system to Gene2 upregulation (2nd iteration). B) Spreading of perturbation on system (3th iteration)

1.2 *minsamp* dataset analysis with interaction factors.

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", title = " ")
```

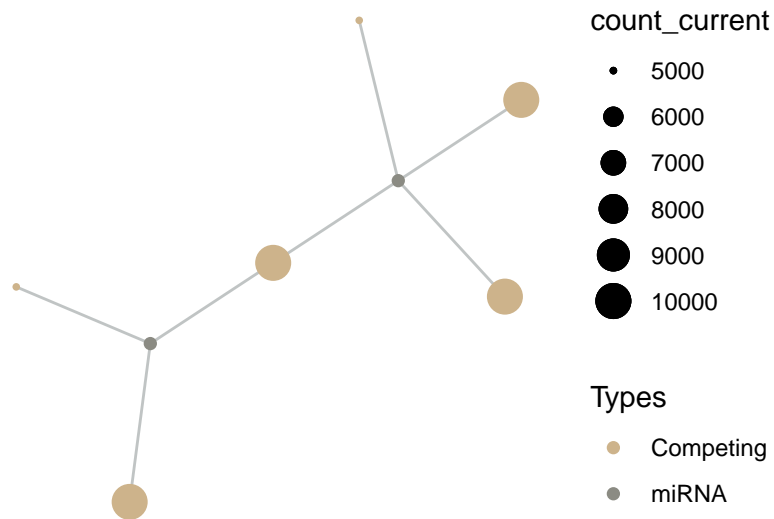


Figure S5: Minimal Dataset with interaction factors in Steady-state

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% update_how("Gene2",
  2) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", title = " ")
```

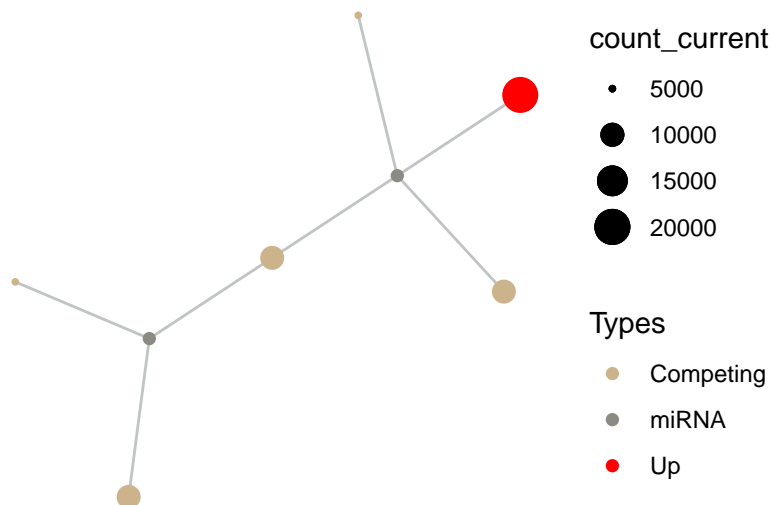


Figure S6: When Gene2 is upregulated on Minimal Dataset with interaction factors

```
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 = 1) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", Downregulation = "blue",
  title = "A")

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) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", Downregulation = "blue",
  title = "B")
```

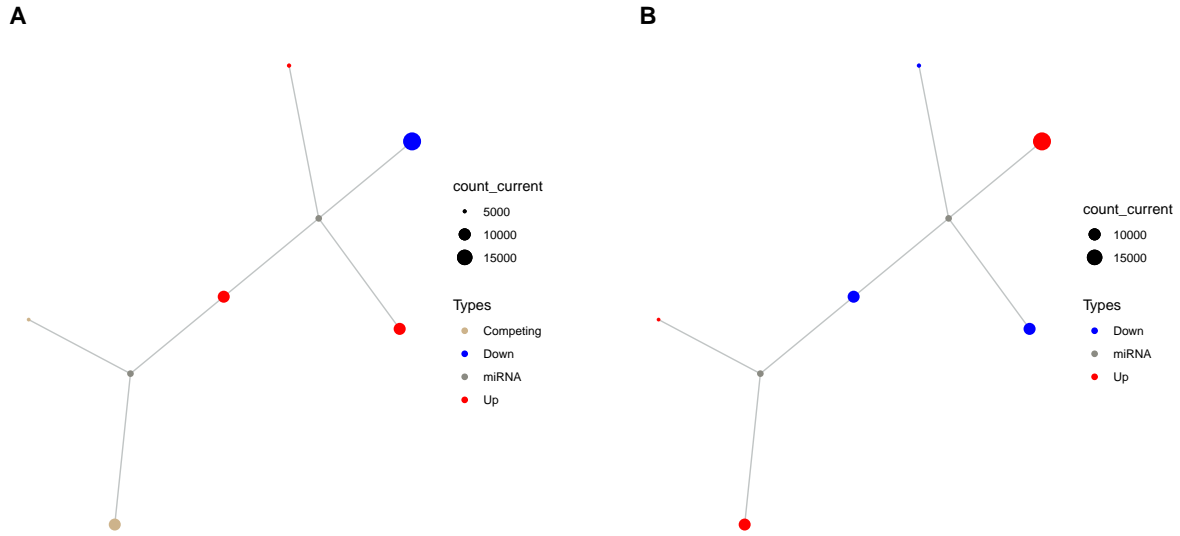


Figure S7: Sequential iteration of minsamp data with interaction factors A)First response of system to Gene2 upregulation (2nd iteration). B)Spreading of perturbation on system (3th iteration)

1.3 Common target perturbation in *minsamp* dataset.

```
priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", title = "A")

priming_graph(minsamp, competing_count = Competing_expression,
  miRNA_count = miRNA_expression, aff_factor = c(energy,
    seed_type), deg_factor = region) %>% update_how("Gene4",
  2) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", title = "B")

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 = 1) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", title = "C")

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) %>% vis_graph(Competing_color = "navajowhite3",
  mirna_color = "ivory4", Upregulation = "red", title = "D")
```

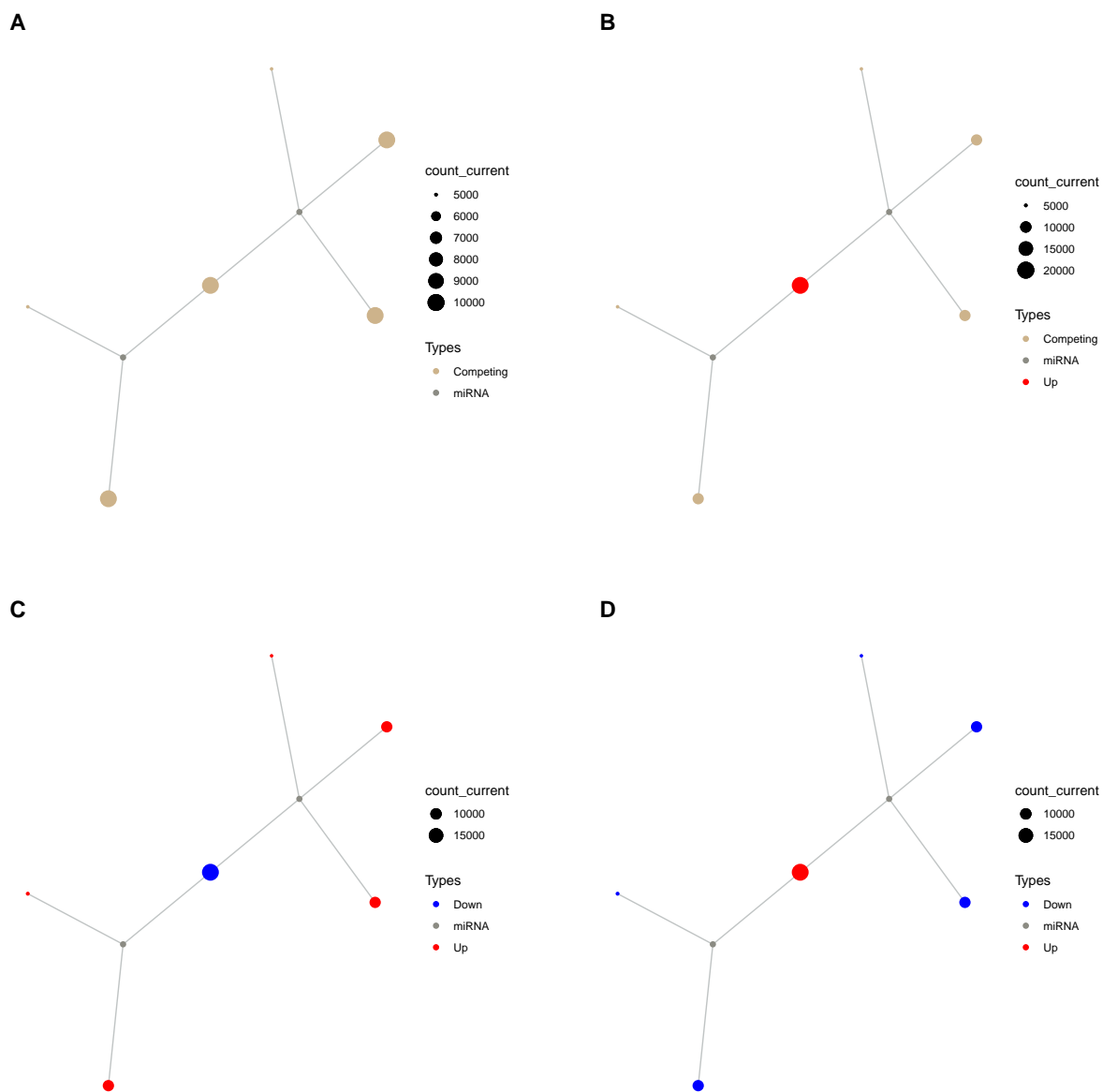



Figure S8: Perturbation of Gene4 on minsamp data with interaction factors. A) Network at steady-state. B) Upregulation of Gene4. C) Primary response of network to upregulation of Gene4. D) Re-regulation of whole nodes on system (3th iteration)

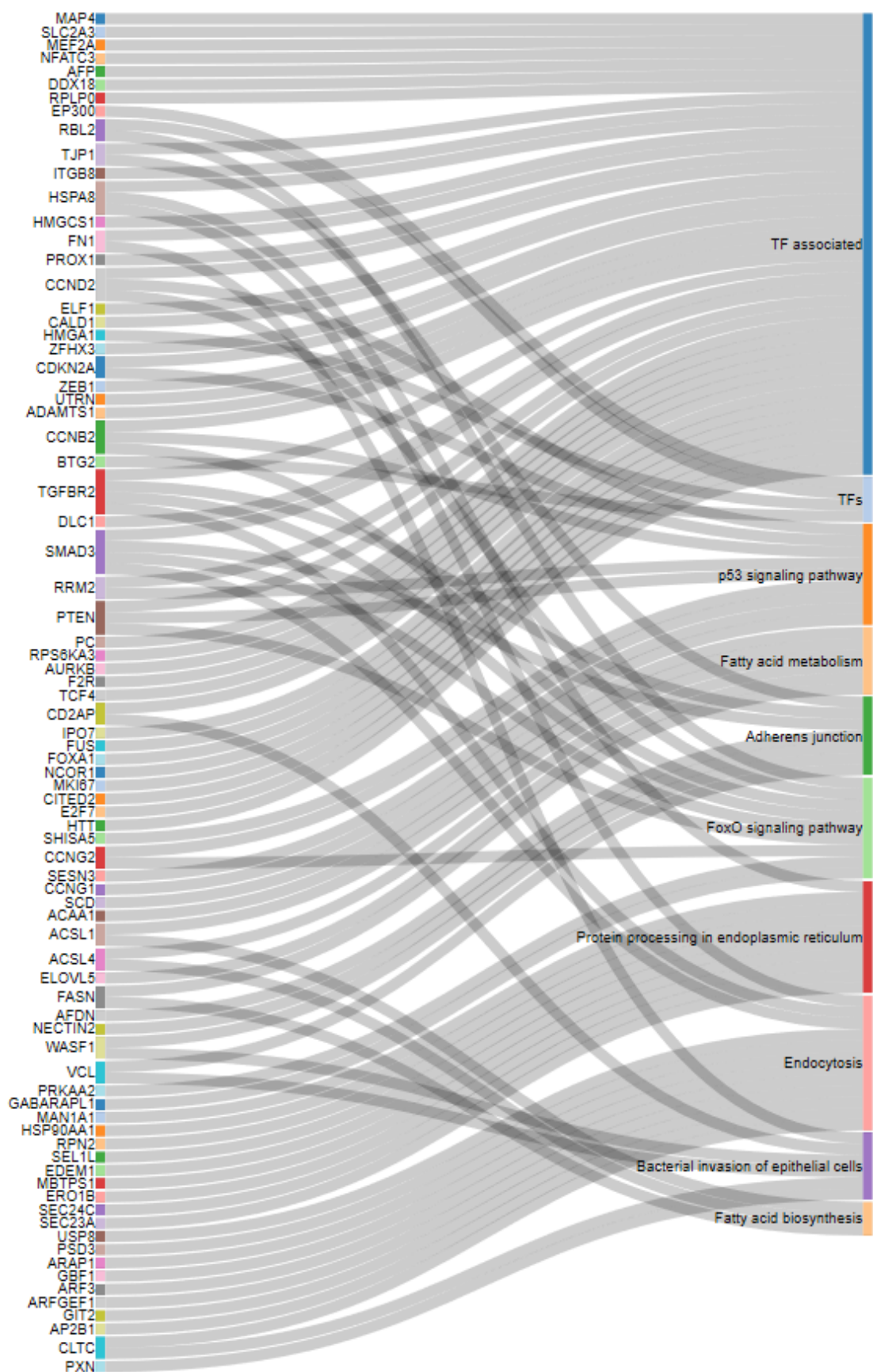


Figure S9: Gene analysis of transcription factor association and KEGG pathway, obtained from experimental miRNA:target dataset

2 Supplementary Tables

2.1 *minsamp* dataset

Table S1: *minsamp* sample dataset that includes interaction factors. The parameters which affect miRNA:target interactions (i.e. seed type, region, energy) are provided in sample dataset.

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

2.2 Significant factors in miRNA:target interactions

Some of information about miRNA:target interactions were exhibited directly by high-throughput studies. On the other hand, we were examined other interaction factors based on different studies.

- (Helwak et al. 2013; Moore et al. 2015) reported the energy values in miRNA:target interactions.
- Comparisons of canonical seed types were evaluated by study of (Grimson et al. 2007), while functional and non-functional seed interactions were studied by (Bartel 2009) and (Betel et al. 2010).
- Numeric definition of target region location effect was performed based on studies of (Hausser et al. 2013) and (Helwak et al. 2013)

Table S2: Efficiency factors for seed types.

seed type	seed type effect
6-mer_noncanonical	0.05
9-mer	0.43
6-mer	0.07
8-mer	0.43
7-mer	0.23
none	0.01
5-mer_noncanonical	0.04
5-mer	0.05
6-merA1_noncanonical	0.05
7-mer-8m_noncanonical	0.21
7-mer-8m	0.25
8-mer_noncanonical	0.35
7-merA1_noncanonical	0.16
7-merA1	0.19
6-merA1	0.07

Table S3: Efficiency factors for binding regions on targets

region	region effect
3UTR	0.84
CDS	0.42
3UTRCDS	0.93
5UTR	0.01
5UTRCDS	0.42
none	0.01
intron	0.01
CDS3UTR	0.93
CDS5UTR	0.42
exon_unclassified	0.20
CDS3UTRintron	0.93
3UTRintron	0.84
CDSintron	0.42
5UTRintron	0.01
5UTR3UTR	0.93
CDS5UTR3UTR	0.93

2.3 Content of High-throughput experimental studies

Table S4: miRNA:target pairs supported by High-throughput Experiments

Variable	Structure	Means
cluster	character	Barcode from experimentally method
chromosome	character	Chromosome of Target gene from raw data
start_position	numeric	Gene start position from raw data
end_position	numeric	Gene end position from raw data
strand	character	Gene strand
hgnc_symbol	character	Gene name (Symbol)
Ensembl_Gene_Id	character	Ensembl Gene Id of gene
Ensembl_Transcript_Id	character	Ensembl transcript id of mRNA of Target gene
target_seq	character	mRNA sequences targeted by miRNA
miRNA	character	miRNA id (from miRBase version 21)
miR_seq	character	miRNA sequence
seed_type	character	seed type of miRNA:target interaction
Energy	numeric	Energy of miRNA:target binding
HG38build_loc	character	Recent chromosomal location of Gene

Variable	Structure	Means
Genome_build	character	Genome build of given chromosome, start and end positions
region	character	interaction location on target
region_effect	numeric	Coefficient of location on target
seed_type_effect	numeric	Coefficient for seed sequence of miRNA:target interaction

2.4 Variables of network object during simulation

As a result of simulation a dataset, a graph object is obtained that includes various variables in edge and node data. A graph object includes variables at following.

Table S5: The context graph object during the process.

Variables	Structure	Means
<i>Node Variables</i>		
name	character	node name
type	character	Competing or miRNA
node_id	numeric	in on graph object
initial_count	numeric	Initial Expression value of node
count_pre	numeric	Expression value of node at previous regulation
count_current	numeric	Existing expression value of node
changes_variable	character	Regulation of node (Up, down or steady)
<i>Edge Variables</i>		
Competing name	character	name of genes
miRNA name	character	name of miRNAs
Competing expression	numeric	Expression values of competing elements at steady-state
miRNA expression	numeric	Expression values of miRNA elements at steady-state
energy	numeric	coefficient of miRNA:target interactions (binding affinity)
seed type	numeric	coefficient of miRNA:target interactions (binding affinity)
region	numeric	coefficient of miRNA:target interactions (degradation efficiency)
aff factor	numeric	coefficient scaled and combined affinity factor
degg factor	numeric	coefficient scaled and combined degradation factor
comp_count_list	list	list of competing expression for each iteration
comp_count	numeric	pre: competing expression at previous iteration; current: competing expression at present iteration
mirna_count_list	list	list of miRNA expression for each iteration
mirna_count	numeric	pre: miRNA expression at previous iteration; current: miRNA expression at present iteration
effect	numeric	pre: total miRNA repressive effect on individual target at previous iteration ; current: miRNA repressive effect on individual target at present iteration
effect_list	list	list of miRNA repressive effect on individual target for each iteration

REFERENCES

- Bartel, David P. 2009. “MicroRNAs: Target Recognition and Regulatory Functions.” *Cell* 136 (2): 215–33. <https://doi.org/10.1016/j.cell.2009.01.002>.
- Betel, Doron, Anjali Koppal, Phaedra Agius, Chris Sander, and Christina Leslie. 2010. “Comprehensive Modeling of microRNA Targets Predicts Functional Non-Conserved and Non-Canonical Sites.” *Genome Biology* 11 (8): R90.
- Grimson, Andrew, Kyle Kai-How Farh, Wendy K. Johnston, Philip Garrett-Engele, Lee P. Lim, and David P. Bartel. 2007. “MicroRNA Targeting Specificity in Mammals: Determinants Beyond Seed Pairing.” *Molecular Cell* 27 (1): 91–105. <https://doi.org/10.1016/j.molcel.2007.06.017>.
- Hausser, J., A. P. Syed, B. Bilen, and M. Zavolan. 2013. “Analysis of CDS-Located miRNA Target Sites Suggests That They Can Effectively Inhibit Translation.” *Genome Research* 23 (4): 604–15. <https://doi.org/10.1101/gr.139758.112>.
- Helwak, Aleksandra, Grzegorz Kudla, Tatiana Dudnakova, and David Tollervey. 2013. “Mapping the Human miRNA Interactome by CLASH Reveals Frequent Noncanonical Binding.” *Cell* 153 (3): 654–65. <https://doi.org/10.1016/j.cell.2013.03.043>.
- Moore, Michael J., Troels K. H. Scheel, Joseph M. Luna, Christopher Y. Park, John J. Fak, Eiko Nishiuchi, Charles M. Rice, and Robert B. Darnell. 2015. “miRNA-Target Chimeras Reveal miRNA 3'-End Pairing as a Major Determinant of Argonaute Target Specificity.” *Nature Communications* 6 (November): 8864. <https://doi.org/10.1038/ncomms9864>.