Supplementary Figures and Tables

Selcen Ari   Alper Yilmaz

2020-08-13

Contents

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 = " ")
```
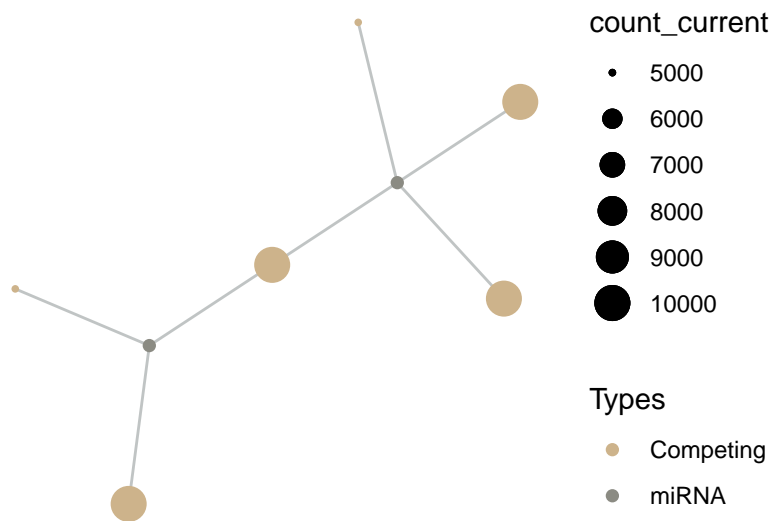
Figure S2: Minimal Dataset in Steady-state

```
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")
```

A

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 = " ")
```
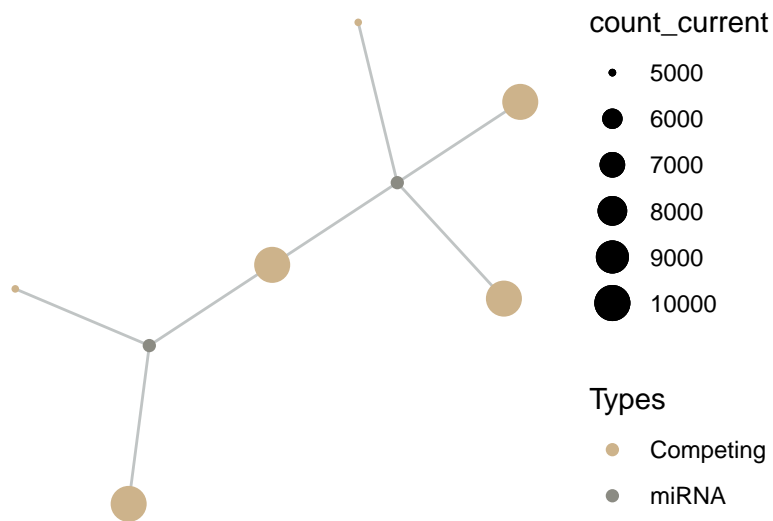
Figure S5: Minimal Dataset with interaction factors in Steady-state

```
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 = " ")
```
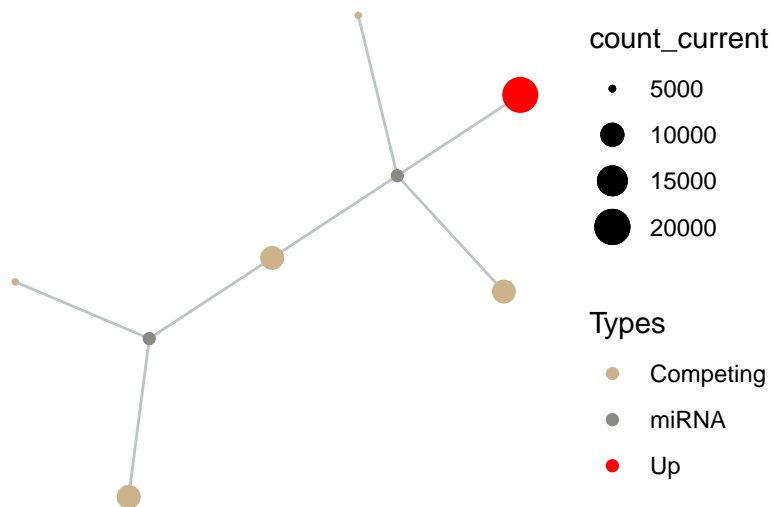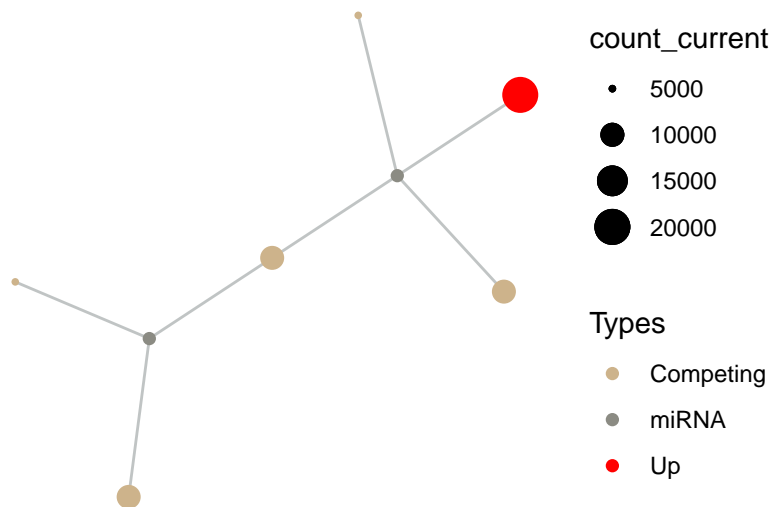
Figure S6: When Gene2 is upregulated on Minimal Dataset with interaction factors

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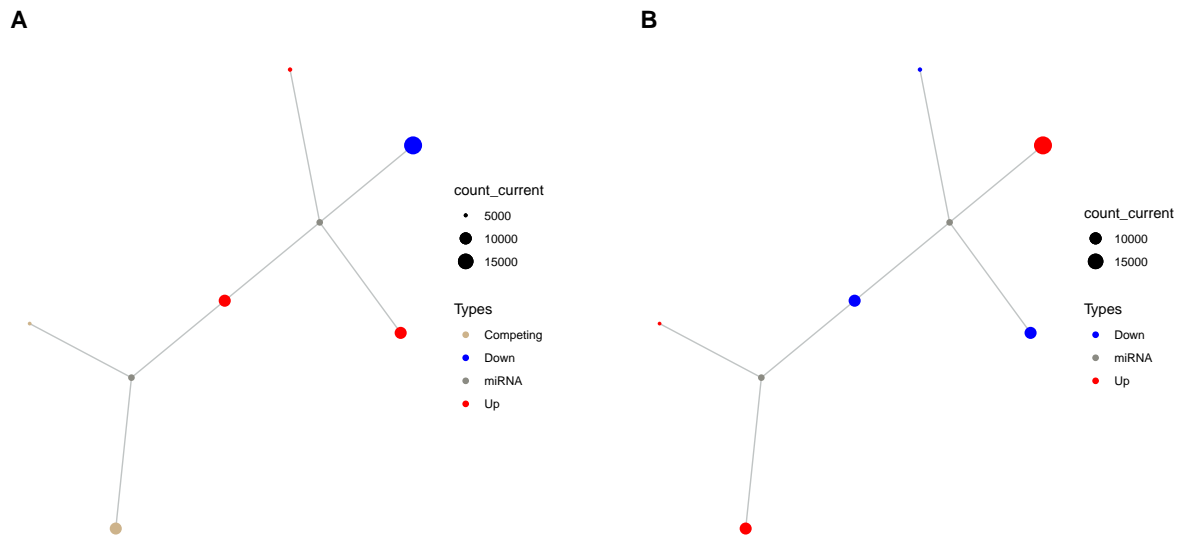
Figure S7: Sequential iteration of minsamp data with interaction factors A)First response of system to Gene2 upregulation (2nd iteration). B)Spreading of perturbation on system (3th iteration)

1.3 Common target perturbation in *minsamp* dataset.

```r
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")
```
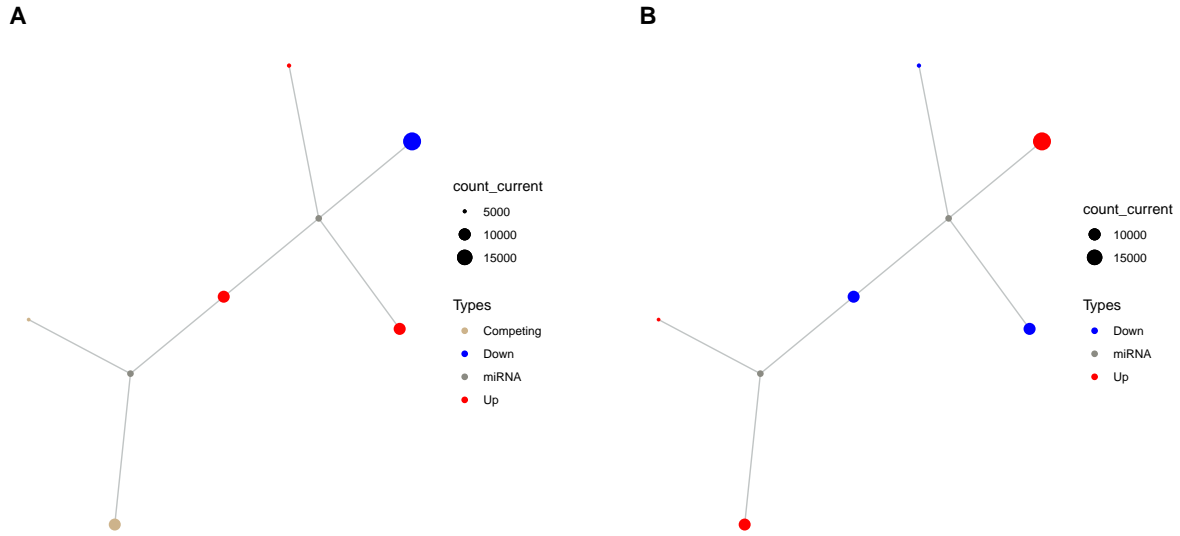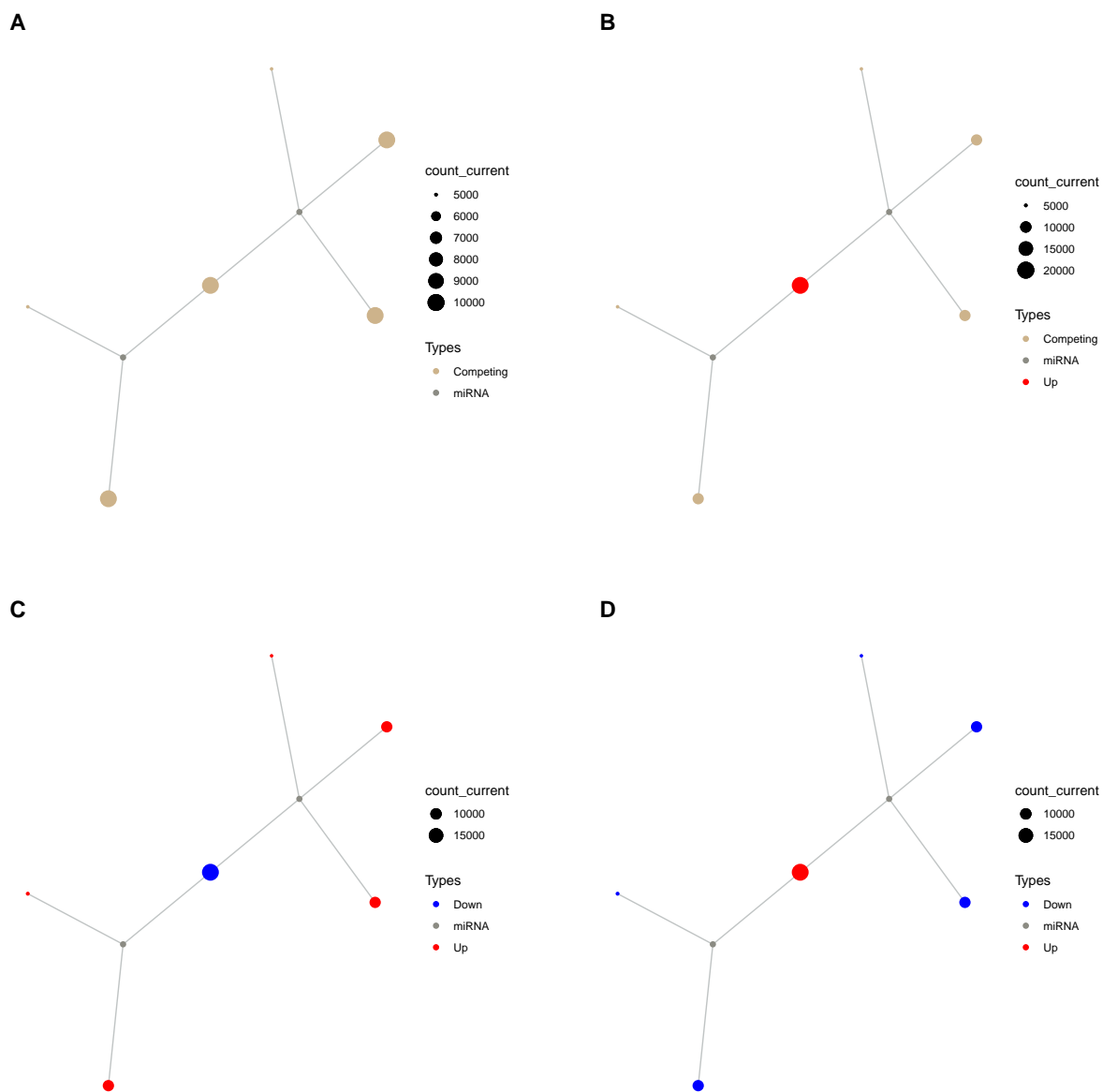
Figure S8: Perturbation of Gene4 on minsamp data with interaction factors. A) Network at steady-state. B) Upregulation of Gene4. C) Primary response of network to upregulation of Gene4. D) Re-regulation of whole nodes on system (3th iteration)
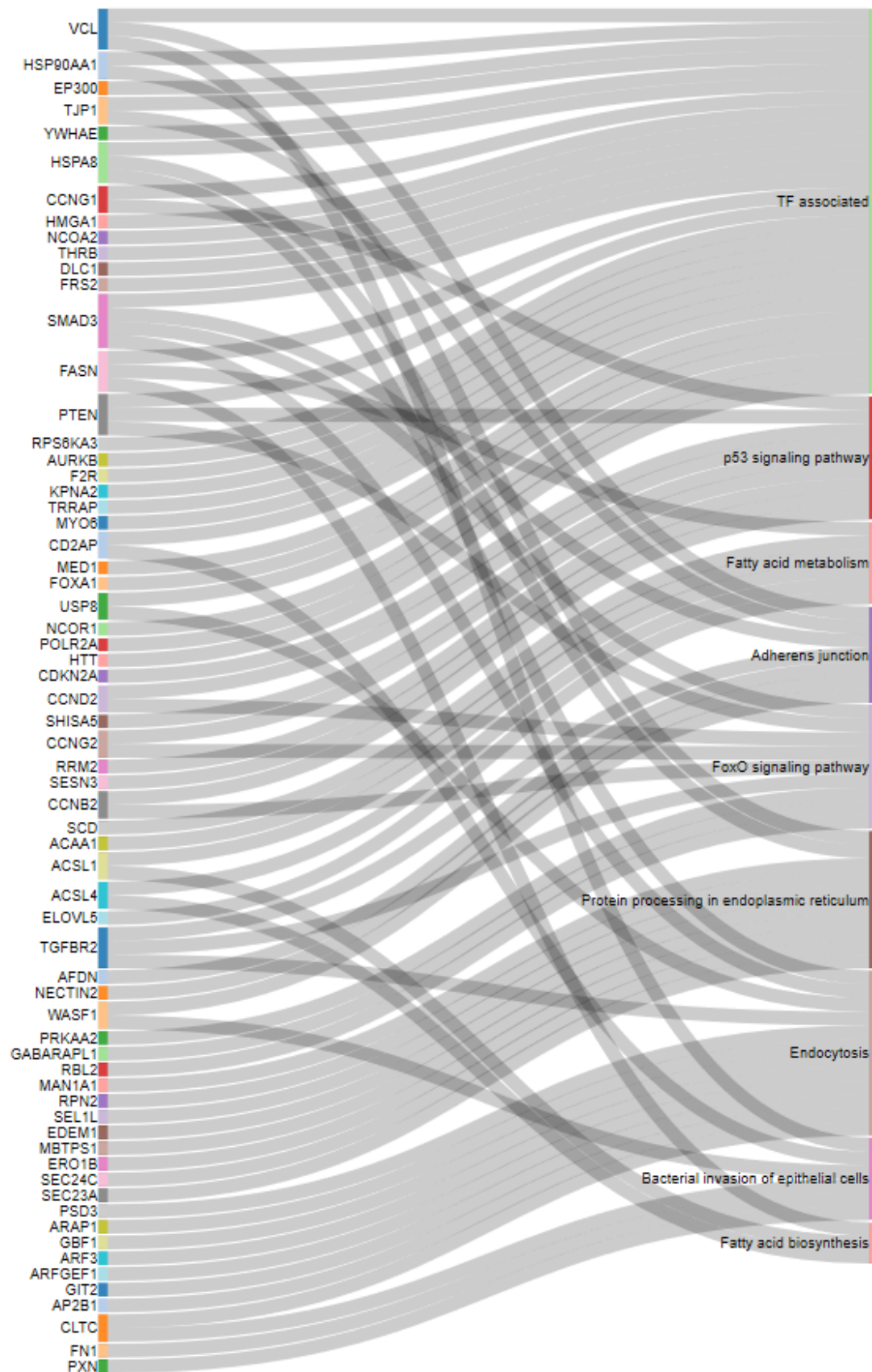
Figure S9: Gene analysis of transcription factor association and KEGG pathway, obtained from experimental miRNA:target dataset

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 interacion 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
Node Variables		
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)
Edge Variables		
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)
afff 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 reppressive effect on individual target at previous iteration ; current: miRNA reppressive effect on individual target at present iteration
effect_list	list	list of miRNA reppressive 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.