

# Supplementary Materials And Methods

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The functions that are used in the this study are developed with R and are available in [Bioconductor](#).

## 1. Determination of perturbation efficiencies of nodes in network.

```
library(ceRNAetsim)
```

## Node efficiencies on Sample+ network

```
## # A tibble: 8 x 3
##   name perturbation_efficiency perturbed_count
##   <chr>          <dbl>          <dbl>
## 1 Gene1          0.132            2
## 2 Gene2          0.198            3
## 3 Gene3          0.0555           2
## 4 Gene4          0.197            4
## 5 Gene5          0.143            1
## 6 Gene6          0.131            1
## 7 Mir1           0.806            3
## 8 Mir2           2.80            3
```

## 2. Additional data manipulation steps

### 2.1 Arrangement of CLASH dataset

CLASH dataset was retrieved from [PubMed](#) (Helwak et al. 2013).

```
clashelwak <- read.table("mmc1.txt", comment.char = "#",
  header = TRUE, skip = 1, stringsAsFactors = FALSE)
```

```
# hg19
```

#### Query of Human Genome 19.

Human genome 19 information was handled through biomaRt package.

```
# HG19
listEnsemblArchives()
listMarts(host = "http://grch37.ensembl.org")
ensemblgrch37 = useMart(host = "http://grch37.ensembl.org",
  biomaRt = "ENSEMBL_MART_ENSEMBL", dataset = "hsapiens_gene_ensembl")
hg19 <- getBM(attributes = c("ensembl_transcript_id",
  "ensembl_gene_id", "chromosome_name", "start_position",
  "end_position", "hgnc_symbol", "entrezgene_id",
  "strand"), mart = ensemblgrch37)
```

#### Adding miRNA and gene information

```
clashelwak <- clashelwak %>% separate(microRNA_name,
  c("Barcode", "Database", "mirna_name", "type"),
  sep = "_") %>% separate(mRNA_name, c("Ensembl_Gene_Id",
  "Ensembl_Transcript_Id", "Hugo_Symbol", "mRNA_Type"),
  sep = "_")
```

MiRNA releases are obtained from [miRBase](#). In this step, release 21 (in Human genome 38) was downloaded.

```

mirbasehg38 <- read.table("mirbasehg38.txt", comment.char = "#") %>%
  filter(V3 != "miRNA_primary_transcript") %>% separate(V9,
    c("ID", "Alias", "Name", "Precursor"), sep = ";") %>%
  mutate(ID = substr(ID, 4, length(ID)), Alias = substr(Alias,
    7, length(Alias)), Name = substr(Name, 6, length(Name)),
    Precursor = substr(Precursor, 14, length(Precursor))) %>%
  dplyr::select(chr = V1, start = V4, end = V5, strand = V7,
    ID, Alias, Name, Precursor)

```

CLASH dataset is published in miRBase release 15 and Human Genome 19 version.

```

clashelwakfinal <- read_tsv("mirna_mature.txt", col_names = FALSE) %>%
  filter(startsWith(X2, "hsa")) %>% dplyr::select(mirna_ID = X2,
    mirbase_ID = X3) %>% inner_join(mirbasehg38 %>%
  dplyr::select(ID, Name), by = c(mirbase_ID = "ID")) %>%
  dplyr::select(mirbase_ID, Name) %>% distinct() %>%
  inner_join(clashelwak, by = c(mirbase_ID = "Barcode")) %>%
  dplyr::select(Name, miRNA_seq, Ensembl_Gene_Id,
    Ensembl_Transcript_Id, Hugo_Symbol, mRNA_seq_extended,
    chimeras_decompressed, seed_type, seed_basepairs,
    folding_class, seq_ID, folding_energy, X5.UTR,
    CDS, X3.UTR) %>% inner_join(hg19, by = c(Ensembl_Gene_Id = "ensembl_gene_id",
    Ensembl_Transcript_Id = "ensembl_transcript_id",
    Hugo_Symbol = "hgnc_symbol")) %>% mutate(region1 = ifelse(X5.UTR ==
    "1", "5UTR", " "), region2 = ifelse(X3.UTR == "1",
    "3UTR", " "), region3 = ifelse(CDS == "1", "CDS",
    " ")) %>% unite(region, c(region1, region2, region3),
    sep = "||") %>% dplyr::select(chromosome_name,
    start_position, end_position, strand, Hugo_Symbol,
    Ensembl_Gene_Id, Ensembl_Transcript_Id, mRNA_seq_extended,
    Name, miRNA_seq, seq_ID, seed_type, seed_basepairs,
    folding_class, folding_energy, region) %>% as_tibble()

```

## Converting CLASH data to human genome 38 build.

There are different liftover methods for conversion among Human Genome builds. We preferred to use [UCSC liftover tool](#)

```

# Obtaining chromosomal locations from miRNA:target
# interaction dataset.

lift19 <- clashelwakfinal %>% dplyr::select(1, 2, 3) %>%
  unite(start_end, c("start_position", "end_position"),
    sep = "--") %>% mutate(Chromosome = paste0("chr",
    chromosome_name, "")) %>% unite(chromosome_name,
    c("Chromosome", "start_end"), sep = ":")

write_tsv(lift19, "lift19.txt")

# After we searched this file in UCSC browser, the
# output loaded (lift19_del deleted regions on the
# HG38 genome build; hg38clashcomp new locations of

```

```

# the genes on HG38)

lift19_del <- read_tsv("deleted_lift19.txt")
colnames(lift19_del)[1] <- "chromosome_loc"

lift19_del <- lift19_del %>% dplyr::filter(startsWith(chromosome_loc,
  "chr")) %>% separate(chromosome_loc, c("Chr", "End"),
  "-", remove = TRUE) %>% separate(Chr, c("Chr",
  "Start"), ":", remove = TRUE)

lift19_del$Start <- as.numeric(lift19_del$Start)
lift19_del$End <- as.numeric(lift19_del$End)

# removing deleted location from CLASH dataset

clashelwakfinal <- clashelwakfinal %>% mutate(Chromosome = paste0("chr",
  chromosome_name, "")) %>% dplyr::anti_join(lift19_del,
  by = c(Chromosome = "Chr", start_position = "Start",
  end_position = "End"))

hg38clash <- read.delim("hg38clashcomp.txt", header = FALSE,
  stringsAsFactors = FALSE)

# adding new location information:

clashelwakfinal <- clashelwakfinal %>% bind_cols(hg38clash)

colnames(clashelwakfinal)[18] <- "HG38build_loc"

clashelwakfinal <- clashelwakfinal %>% dplyr::mutate(Genom_build = rep("hg19"))

# Arrangement in dataset

clashelwakfinal <- clashelwakfinal %>% dplyr::select(cluster = seq_ID,
  chromosome = Chromosome, start_position, end_position,
  strand, hgnc_symbol = Hugo_Symbol, Ensembl_Gene_Id,
  Ensembl_Transcript_Id, target_seq = mRNA_seq_extended,
  miRNA = Name, miR_seq = miRNA_seq, seed_type, seed_type2 = seed_basepairs,
  seed_type3 = folding_class, Energy = folding_energy,
  HG38build_loc, Genom_build, region)

clashelwakfinal$strand <- as.character(clashelwakfinal$strand)

str(clashelwakfinal)

```

## Interpreting the CLASH seed structures in dataset

```

clashelwakfinal <- clashelwakfinal %>% mutate(seed_type = ifelse(seed_type ==
  "noncanonical_seed" & seed_type2 > 4 & seed_type3 ==
  "I", paste0(seed_type2, "-mer"), seed_type), seed_type = ifelse(seed_type ==
  "noncanonical_seed" & seed_type2 > 4 & seed_type3 ==

```

```

"II", paste0(seed_type2, "-mer_noncanonical"),
seed_type), seed_type = ifelse(seed_type == "noncanonical_seed" &
seed_type2 > 4 & seed_type3 == "III", paste0(seed_type2,
"-mer_noncanonical"), seed_type), seed_type = ifelse(seed_type ==
"noncanonical_seed" & seed_type2 > 4 & seed_type3 ==
"IV", paste0(seed_type2, "-mer_noncanonical"),
seed_type), seed_type = ifelse(startsWith(seed_type,
"no"), "none", seed_type)) %>% dplyr::select(-seed_type2,
-seed_type3)

```

## 2.2 Arrangement of CLEAR-CLiP Dataset (Moore et al. 2015)

CLASH dataset was retrieved from [Nature article](#)

```

clearclip <- read_xlsx("CLEAR-CLIP.xlsx")

# Clearclip hg18

```

### Query of Human Genome 18

```

# HG18
listEnsemblArchives()
listMarts(host = "may2009.archive.ensembl.org")
ensembl54 = useMart(host = "may2009.archive.ensembl.org",
  biomart = "ENSEMBL_MART_ENSEMBL", dataset = "hsapiens_gene_ensembl")

hg18 <- getBM(attributes = c("ensembl_transcript_id",
  "ensembl_gene_id", "chromosome_name", "start_position",
  "end_position", "hgnc_symbol", "entrezgene", "strand"),
  mart = ensembl54)

```

### Adding Genome Information to dataset

```

clearclipfinal <- hg18 %>% inner_join(clearclip, by = c(entrezgene = "gene.id",
  hgnc_symbol = "gene.symbol")) %>% distinct()

```

### Converting human genome build

```

# Obtaining chromosomal locations from miRNA:target
# interaction dataset.

lift18 <- clearclipfinal %>% unite(start_end, c("start_position",
  "end_position"), sep = "-") %>% unite(location,
  c("chr", "start_end"), sep = ":") %>% dplyr::select(location)

write_tsv(lift18, "lift18.txt")

```

```

# After we searched this file in UCSC browser, the
# output loaded (deleted_lift18 deleted regions on
# the HG38 genome build; hg38clearclip new
# locations of the genes on HG38)

deleted_lift18 <- read_tsv("deleted_lift18.txt")

colnames(deleted_lift18)[1] <- "Chromosome_loc"

deleted_lift18 <- deleted_lift18 %>% dplyr::filter(startsWith(Chromosome_loc,
  "chr")) %>% separate(Chromosome_loc, c("Chr", "End"),
  "-", remove = TRUE) %>% separate(Chr, c("Chr",
  "Start"), ":", remove = TRUE)

deleted_lift18$Start <- as.numeric(deleted_lift18$Start)
deleted_lift18$End <- as.numeric(deleted_lift18$End)

# removing deleted location from CLEAR-CLiP dataset

clearclipfinal <- clearclipfinal %>% dplyr::anti_join(deleted_lift18,
  by = c(chr = "Chr", start_position = "Start", end_position = "End"))

hg38clearclip <- read.delim("hg38clearclip.txt", header = FALSE,
  stringsAsFactors = FALSE)

clearclipfinal <- clearclipfinal %>% bind_cols(hg38clearclip)

colnames(clearclipfinal)[28] <- "HG38build_loc"

# adding new location information:

clearclipfinal <- clearclipfinal %>% dplyr::mutate(Genom_build = rep("hg18"))

# Arrangement in dataset

clearclipfinal <- clearclipfinal %>% dplyr::select(cluster = cluster.ID,
  chromosome = chr, start_position, end_position,
  strand = strand.y, hgnc_symbol, Ensembl_Gene_Id = ensembl_gene_id,
  Ensembl_Transcript_Id = ensembl_transcript_id,
  target_seq = target.map, miRNA, miR_seq = miR.map,
  seed_type = "seed match", Energy = MFE, HG38build_loc,
  Genom_build, region)

```

## Seed type manipulation in CLEAR-CLiP dataset

In CLEAR-CLiP dataset, seed types were shown in detail. We adjusted as canonical and non-canonical.

```

clipdata_seed <- data_frame(seed_type = c("5mer_1",
  "5mer_2", "5mer_3", "6mer", "6mer.indel", "6mer.mm",
  "6mer_off.mm", "6merA1", "6merA1.indel", "6merA1.mm",
  "7merA1", "7merA1.indel", "7merA1.mm", "7merm8",
  "7merm8.indel", "7merm8.mm", "8mer", "8mer.indel",

```

```

"8mer.mm", "NA"), seed_type_com = c("5-mer", "5-mer_noncanonical",
"5-mer_noncanonical", "6-mer", "6-mer_noncanonical",
"6-mer_noncanonical", "6-mer_noncanonical", "6-merA1",
"6-merA1_noncanonical", "6-merA1_noncanonical",
"7-merA1", "7-merA1_noncanonical", "7-merA1_noncanonical",
"7-mer-8m", "7-mer-8m_noncanonical", "7-mer-8m_noncanonical",
"8-mer", "8-mer_noncanonical", "8-mer_noncanonical",
"none"))

clearclipfinal <- clearclipfinal %>% inner_join(clipdata_seed,
  by = "seed_type") %>% dplyr::select(1:11, seed_type = seed_type_com,
  Energy, HG38build_loc, Genom_build, region)

```

## 2.3 Integration of two experimental dataset

```

experimentalmirnagene <- bind_rows(clashelwakfinal,
  clearclipfinal) %>% distinct()

```

### Adding Coefficients of Interaction factors

Energy values in miRNA:target pairs are represented by high-throughput studies (Helwak et al. 2013; Moore et al. 2015) which are utilized in this study. On the other hand, we have specified the other interaction factors, seed type and location of binding region on the target, as numeric values based on the previous studies. (Grimson et al. 2007) have compared the seed types' effect on target repression with few miRNA had canonical seed pairing in their study. Additionally, (Bartel 2009) and (Betel et al. 2010) have studied on functional and non-functional seed interactions. Based on results of these studies we have arranged seed types of miRNA:target interactions as numeric values. We also have redefined location of binding region on the target as numeric values, based on studies of (Hausser et al. 2013) and (Helwak et al. 2013). With this process, we have handled this integrated dataset in context of competitor behaviors and functionality of interactions.

In this step we added numeric intraction values at followings

Fistly, we organized these values due to the fact that the regions were defined differently in two datasets. After that, region effect was added as numeric values (shown in Table S3).

```

experimentalmirnagene <- experimentalmirnagene %>%
  mutate(region2 = str_replace_all(region, "NA",
    ""), region3 = str_replace_all(region2, "\\|",
    ""), region = str_replace_all(region3, c('3'UTR' = "3UTR",
    '5'UTR' = "5UTR")))) %>% dplyr::select(-region2,
  -region3) %>% mutate(region_effect = as.double(ifelse(region %in%
  c("3UTRCDS", "CDS3UTR", "5UTR3UTR", "CDS5UTR3UTR",
    "CDS3UTRintron"), "0.93", ifelse(region %in%
  c("CDS", "CDSintron"), "0.42", ifelse(region %in%
  c("3UTR", "3UTRintron"), "0.84", ifelse(region %in%
  c("5UTR", "5UTRintron"), "0.01", ifelse(region %in%
  c("5UTRCDS", "CDS5UTR"), "0.42", ifelse(region %in%
  c("intron", ""), "0.01", ifelse(region %in% c("exon_unclassified",
    ""), "0.2", NA))))))))))

```

Secondly, we organized seed type interactions in *Seed type manipulation* section for CLEAR-CLiP dataset to show as found in CLASH dataset. Same type formatted values added dataset as numeric values (shown in Table S2).

```
seed_type_effect <- data_frame(seed_type = c("5-mer",
      "5-mer_noncanonical", "6-mer", "6-mer_noncanonical",
      "6-merA1", "6-merA1_noncanonical", "7-mer", "7-mer_noncanonical",
      "7-merA1", "7-merA1_noncanonical", "7-mer-8m",
      "7-mer-8m_noncanonical", "8-mer", "8-mer_noncanonical",
      "9-mer", "9-mer_noncanonical", "none"), seed_type_effect = c(0.05,
      0.04, 0.07, 0.05, 0.07, 0.05, 0.23, 0.19, 0.19,
      0.16, 0.25, 0.21, 0.43, 0.35, 0.43, 0.35, 0.01))
experimentalmirnagene <- experimentalmirnagene %>%
  inner_join(seed_type_effect, by = "seed_type")

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

```
## # A tibble: 45,340 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
## 7 L1HS-4~ chr11            134123389     134135749 1      ACAD8
## 8 0727A~~ chr15             59397277     59417244 1      CCNB2
## 9 L2HS-1~ chr19             37001597     37019562 -1     ZNF260
## 10 L2HS-9~ chr11            64889252     64902004 -1     SYVN1
## # ... with 45,330 more rows, and 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 context of dataset is shown in Table S5 in Supplementary Tables.

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- 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>.



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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>.