# Package 'CeRNASeek'

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Title Identification and Analysis of ceRNA Regulation

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Description Provides several functions to identify and analyse miRNA sponge, including popular methods for identifying miRNA sponge interactions, two types of global ceRNA regulation prediction methods and four types of context-specific prediction methods( Li Y et al.(2017) <doi:10.1093/bib/bbx137>), which are based on miRNA-messenger RNA regulation alone, or by integrating heterogeneous data, respectively. In addition, For predictive ceRNA relationship pairs, this package provides several downstream analysis algorithms, including regulatory network analysis and functional annotation analysis, as well as survival prognosis analysis based on expression of ceRNA ternary pair.

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CeRNASeek-package CeRNASeek

### Description

identifying miRNA sponge interactions .

#### Details

CeRNASeek considers five method to identify ceRNA crosstalk, including two types of global ceRNA regulation prediction methods(ratio based, we termed ratio and hypergeometric test based, termed HyperT) and three types of context-specific prediction methods (hypergeometric test plus coexpression, termed HyperC, sensitivity correlation-based method (SC)and conditional mutual information (CMI)-based methods and cernia method). Ratio-based prediction: this method ranked the candidate genes by the proportion of common miRNAs,For instance, we need to identify the ceRNA partners for gene i from all candidate gene sets S, and the ratio is calculated as the intersection of miRNAi and miRNAj divided by miRNAj Where miRNAi is the miRNA set that regulated gene i and miRNAj is the miRNA set that regulated gene j.

Hypergeometric test-based prediction-HyperT: it is usually used the hypergeometric to evaluate whether two genes were coregulated by miRNAs, This statistic test computed the significance of common miRNAs for each RNA pairs.

Hypergeometric test combined with coexpression-based prediction-HyperC: To discover the active ceRNA-ceRNA regulatory pairs in a specific context, the commonly used method is to using the coexpression principle to filter the ceRNA-ceRNA regulation identified based on the above two global methods, this method integrated context-specific gene expression profile data sets. The Pearson correlation coefficient (R) of each candidate ceRNA regulatory pairs identified was calculated.

SC-based prediction: Another method introduced the expression profile data of shared miRNAs, and uses partial correlation to calculate ceRNA interaction pairs. Then, the Sensitivity correlation(SC) of miRNA-M, for the corresponding candidate ceRNA pair is calculated.

CMI-based methods: CMI is widely used to identify the RNA-RNA correlations, given the value of miRNAs. Hermes is a widely used method, which predicts ceRNA interactions from expression profiles of candidate RNAs and their common miRNA regulators using CMI, as in Sumazin et al.Firstly computed the significance of difference of I(miRNAi;T2|T1) and I(miRNAi;T2),where miRNAi represents the ith miRNA in the miRNA shared by the two target genes. Then Shuffled condition target's expression in 1000 times. The final significance based on Fisher's method was calculated.For more information, please refer to the article by Sumazin P et al.

cernia method cernia method is implemented based on the following seven scores: 1. The fraction of common miRNAs; 2. The density of the MREs for all shared miRNAs; 3. The distribution of MREs of the putative ceRNAs; 4. The relation between the overall number of MREs for a putative ceRNA, compared with the number of miRNAs that yield these MREs; 5. The density of the hybridization energies related to MREs for all shared miRNAs; 6. The DT-Hybrid recommendation scores; and 7. The pairwise Pearson correlation between putative ceRNA expressions from selected tissue.

#### References

Xu J, Feng L, Han Z, et al. Extensive ceRNA–ceRNA interaction networks mediated by miRNAs regulate development in multiple rhesus tissues[J]. Nucleic Acids Research, 2016:gkw587.

Sumazin P, Yang X, Chiu H S, et al. An Extensive MicroRNA-Mediated Network of RNA-RNA Interactions Regulates Established Oncogenic Pathways in Glioblastoma[J]. Cell, 2011, 147(2):0-381.

Paci P , Colombo T , Farina L . Computational analysis identifies a sponge interaction network between long non-coding RNAs and messenger RNAs in human breast cancer[J]. BMC Systems Biology, 2014, 8(1):83.

Zhang Y, Xu Y, Feng L, et al. Comprehensive characterization of lncRNA-mRNA related ceRNA network across 12 major cancers[J]. Oncotarget, 2014, 7(39):64148-64167.

Sardina D S, Alaimo S, Ferro A, et al. A novel computational method for inferring competing endogenous interactions[J]. Briefings in Bioinformatics, 2016:bbw084.

ceRNA.app

identify the miRNAs that co-regulate the gene pairs of interest.

### Description

The ceRNA function is used to identify the miRNAs that co-regulate the gene pairs of interest. The users can input the genes of interest. Otherwise, all the paired genes were calculated the number of co-regulating miRNAs.

#### Usage

```
ceRNA.app(miRtar, targetce = NULL)
```

#### Arguments

miRtar	A data frame representing the relationship between miRNA and target. The data frame contains the name of the miRNA and target regulatory relationship.
targetce	a character string (vector) specifying candidate target name to analyse (default (targetce = NULL)).

# Details

Note:All the arguments without default value must be assigned.

A list of identified miRNAs that co-regulate the gene pairs containing following components:

- ceRNA identify the miRNAs that co-regulate the gene pairs of interest, List all possible ceRNA interactions, a 4 columns dataframe as following:
  - targetce represented target names, respectively.
  - anotherce names of modulators that another possible target(modulators) constitutes a ceRNA interaction relation.
  - miRNAs names of miRNA shared by two targets.
  - miRNAs\_num number of miRNAs shared by two targets.
- miR\_1 Number of miRNAs interacting with each target in the input file.

# Examples

##Here we take the regulatory relationship between six genes and 71 miRNAs.

```
ceRNA.app(dataset[["miRtar"]],targetce=NULL)
```

ceRNA.basic

Identifying miRNA sponge interactions using ceRNA.basic function

# Description

This function predicts ceRNA interactions by ratio or hypergeometric test.

# Usage

```
ceRNA.basic(miRtar, targetce = NULL, method = "ratio", numMIR = 1,
cutoff = ifelse(method == "ratio", 1/3, 0.05))
```

#### Arguments

miRtar	A data frame representing the relationship between miRNA and target. The data frame contains the name of the miRNA and target regulatory relationship.
targetce	a character string (vector) specifying candidate target name to analyse (default (targetce = NULL)).
method	a character string (default "ratio") indicating which statistical method to choose to calculate the ceRNA interaction relationship. One of "ratio" (default), or "hypergeometric", can be abbreviated.
numMIR	a numeric vector that specify the minimum number of 2 gene-shared miRNAs
cutoff	a numeric vector of the method("ratio", "hypergeometric") specifying threshold between ceRNA interactions .(default (1/3)).

# Details

Note: All the arguments without default value must be assigned.

#### Value

A list of identified miRNA sponge interactions containing following components:

- cesig predicted significant triplets and related information, a 5 columns dataframe as following:
  - targetce represented target names, respectively.
  - anotherce names of modulators that another target(modulators) constitutes a ceRNA interaction relation.
  - miRNAs names of miRNA shared by two targets.
  - miRNAs\_num number of miRNAs shared by two targets.
  - ratio/pvalue The ratio/pvalue(optional for method("ratio", "hypergeometric")) of the identified ceRNA interaction relation.
- cenotsig predicted not significant triplets and related information, a 5 columns dataframe as following:
  - targetce same as targetce in cesig
  - anotherce same as anotherce in cesig
  - miRNAs same as miRNAs in cesig
  - miRNAs\_num same as miRNAs\_num in cesig
  - pvalue same as pvalue in cesig

#### References

Xu J, Feng L, Han Z, et al. Extensive ceRNA–ceRNA interaction networks mediated by miRNAs regulate development in multiple rhesus tissues[J]. Nucleic Acids Research, 2016:gkw587.

#### Examples

```
##identifying miRNA sponge interactions
##Here we take six candidate targets(modulators) for example
ceRNA.basic(miRtar=dataset[["miRtar"]],targetce=NULL,method="ratio",numMIR = 1,cutoff =1/3)
```

ceRNA.cernia identifying miRNA sponge interactions using ceRNA.cernia function.

# Description

identifying miRNA sponge interactions using ceRNA.cernia.In this function We implement cernia score to identify miRNA sponge interactions. The fraction of common miRNAs; 1. The fraction of common miRNAs; 2. The density of the MREs for all shared miRNAs; 3. The distribution of MREs of the putative ceRNAs; 4. The relation between the overall number of MREs for a putative ceRNA, compared with the number of miRNAs that yield these MREs; 5. The density of the hybridization energies related to MREs for all shared miRNAs; 6. The DT-Hybrid recommendation scores; 7. The pairwise Pearson correlation between putative ceRNA expressions.

#### Usage

#### Arguments

miRtar	A data frame representing the relationship between miRNA and target. The data frame contains the name of the miRNA and target regulatory relationship.
targetce	a character string (vector) specifying candidate target name to analyse (default (targetce = NULL)).
geneexp	An input target expression data frame, the columns are genes and the rows are samples. The expression value may be gene expression ,non-coding RNA expression or expression values of circRNAs and so on.
miRexp	An input miRNA expression data frame, the columns are miRNA and the rows are samples.
mres	miRNA response elements (mres) data frame, each row contains five elements: mirna, target, energy, gap_l, gap_r.
numMIR	a numeric vector that specify the minimum number of 2 gene-shared miRNAs.
cor_cutoff	a numeric vector of the form method specifying threshold between ceRNA interactions correlation,default(cor_cutoff=0).
s_cutoff	the threshold of seven comprehensive scores.

# Details

Note:All the arguments without default value must be assigned.The miRNA in the file of the targetmiRNA regulatory relationship pair should also be present in the expression profile data file. Internal functions (parMM, graphWeights, recommendation, dtHybrid) of cernia method are from the website: https://github.com/dsardina/cernia Copyright 2016 Rosalba Giugno Licensed under the Apache License, Version 2.0 (the 'License')

#### Value

A list of identified miRNA sponge interactions containing following components:

- targetce represented target names, respectively.
- anotherce names of modulators that another target(modulators) constitutes a ceRNA interaction relation.
- miRNAs\_num names of miRNA in the triplet.
- Score 1 The fraction of common miRNAs;
- Score 2 The density of the MREs for all shared miRNAs;
- Score 3 The distribution of MREs of the putative ceRNAs;
- Score 4 The relation between the overall number of MREs for a putative ceRNA, compared with the number of miRNAs that yield these MREs;
- Score 5 The density of the hybridization energies related to MREs for all shared miRNAs;

- Score 6 The DT-Hybrid recommendation scores;
- Score 7 The pairwise Pearson correlation between putative ceRNA expressions.
- Normalized score combine the sum of 7 scores and standardize by z-score.

#### References

Sardina D S, Alaimo S, Ferro A, et al. A novel computational method for inferring competing endogenous interactions[J]. Briefings in Bioinformatics, 2016:bbw084.

# Examples

ceRNA.cmi

identifying miRNA sponge interactions using ceRNA.cmi function.

# Description

identifying miRNA sponge interactions using ceRNA.cmi.In this function We implement CMI methods to identify miRNA sponge interactions.

#### Usage

```
ceRNA.cmi(miRtar, targetce = NULL, geneexp, miRexp, numMIR = 1, num_perm = 100,
cutoff = 0.05)
```

#### Arguments

miRtar	A data frame representing the relationship between miRNA and target. The data frame contains the name of the miRNA and target regulatory relationship.
targetce	a character string (vector) specifying candidate target name to analyse (default (targetce = NULL)).
geneexp	An input target expression data frame, the columns are genes and the rows are samples. The expression value may be gene expression ,non-coding RNA expression or expression values of circRNAs and so on.
miRexp	An input miRNA expression data frame, the columns are miRNA and the rows are samples.
numMIR	a numeric vector that specify the minimum number of 2 gene-shared miRNAs
num_perm	The number of random default(num_perm=100).
cutoff	a numeric vector of the form method specifying threshold between ceRNA interactions default(cutoff=0.05).

### Details

Note:All the arguments without default value must be assigned. The miRNA in the file of the targetmiRNA regulatory relationship pair should also be present in the expression profile data file.

# Value

A list of identified miRNA sponge interactions containing following components:

- ceRNA\_cmi predicted triplets and related information, a 5 columns dataframe as following:
  - targetce represented target names, respectively.
  - miRNA names of miRNA in the triplet.
  - anotherce names of modulators that another target(modulators) constitutes a ceRNA interaction relation.
  - cmi Conditional mutual information(CMI) of triplets calculated using expression values.
  - pvalue The p value of the identified ceRNA interaction relation by CMI.
- ceRNA\_comP Number of miRNAs interacting with each target in the input file.
  - targetce represented target names, respectively.
  - anotherce names of predicted another target(modulators) constitutes a ceRNA interaction relation.
  - commonMiR names of miRNA shared by predicted ceRNA.
  - xsq Chi-square value of p-value pvalue in conditional mutual information .
  - comP The p value calculated by incomplete gamma function .

# References

Sumazin P, Yang X, Chiu H S, et al. An Extensive MicroRNA-Mediated Network of RNA-RNA Interactions Regulates Established Oncogenic Pathways in Glioblastoma[J]. Cell, 2011, 147(2):0-381.

#### Examples

```
ceRNA.cor
```

identifying miRNA sponge interactions using ceRNA.cor function

# Description

identifying miRNA sponge interactions using ceRNA.cor.In this function We implement several popular linear methods (HyperC, SC) to identify miRNA sponge. interactions.

# ceRNA.cor

# Usage

```
ceRNA.cor(miRtar, targetce = NULL, geneexp, miRexp, numMIR = 1, method = "pearson",
numrandom = 100)
```

## Arguments

miRtar	A data frame representing the relationship between miRNA and target. The data frame contains the name of the miRNA and target regulatory relation-ship.Required option for method "pearson" and "partial correlation".
targetce	a character string (vector) specifying candidate target name to analyse (default (targetce = NULL)).
geneexp	An input target expression data frame, the columns are genes and the rows are samples. The expression value may be gene expression ,non-coding RNA expression or expression values of circRNAs and so on. Required option for method pearson" and "partial correlation".
miRexp	An input miRNA expression data frame, the columns are miRNA and the rows are samples. Required option for method pearson" and "partial correlation".
numMIR	a numeric vector that specify the minimum number of 2 gene-shared miRNAs.
method	a character string (default "pearson") indicating which statistical method to choose to calculate the ceRNA interaction relationship. One of "pearson" (default), or "partial correlation", can be abbreviated.
numrandom	The number of random. Required option for method "partial correlation", default (numrandom = $100$ ).

# Details

Note:All the arguments without default value must be assigned.

#### Value

A list of identified miRNA sponge interactions containing following components:

- ceRNA predicted triplets and related information, a 5 columns dataframe as following:
  - targetce represented target names, respectively.
  - anotherce names of modulators that another target(modulators) constitutes a ceRNA interaction relation.
  - miRNAs names of miRNA shared by two targets.
  - miRNAs\_num number of miRNAs shared by two targets.
  - correlation The correlation of the identified ceRNA interaction relation.
  - pvalue The p value of the identified ceRNA interaction relation.
- miR\_1 Number of miRNAs interacting with each target in the input file.

# References

Paci P, Colombo T, Farina L. Computational analysis identifies a sponge interaction network between long non-coding RNAs and messenger RNAs in human breast cancer[J]. BMC Systems Biology, 2014, 8(1):83. Zhang Y, Xu Y, Feng L, et al. Comprehensive characterization of lncRNAmRNA related ceRNA network across 12 major cancers[J]. Oncotarget, 2014, 7(39):64148-64167.

# Examples

```
ceRNA.enrich
```

Enrich the biological functions of interest.

#### Description

Downstream analysis function of ceRNA, This function can be used to identify biological functions of interest, and users can enrich the functions of interest by ceRNA.enrich.

# Usage

```
ceRNA.enrich(data, GOterms, background, threshold = 2, correction = "BH")
```

# Arguments

data	a dataframe that the ceRNA relationship is the data, and the prediction result data obtained according to the ceRNA prediction algorithm.Such as ceRNA.cmi prediction result file.
GOterms	a list that goterm of interest and all gene sets in the term.
background	a vector containing a gene set in which GOterm annotated genes must be. Its id style must be consistent with the id format in <i>GOterms</i> .
threshold	a numeric (default 2) representing min number of intersection between a modu- lator's targets and a GOterms genes.
correction	correction method (default "BH") in one of p.adjust.methods.

#### Details

Note:All the arguments without default value must be assigned.

#### Value

A list of identified miRNA sponge interactions containing following components:

- target represented target names, respectively.
- GOterm the GOterm name.
- target\_num names of represented target in the triplet.
- GOtermnum the gene number of a GOterm;
- term\_tar the number of intersected factor between a GOterm genes and a modulator targets;
- P\_value the p value of the significance enrichment;
- fdr corrected P\_value by the assigned method;

# ceRNA.Net

# Examples

ceRNA.Net	visualize	and	analyze	the	identified	ceRNA-ceRNA	network	using
	ceRNA.N	et fur	nction					

#### Description

A downstream analysis function of ceRNAseekvisualize and analyze the identified ceRNA-ceRNA network, the network can be defined as weighted or un-weighted network.and the basic topological features (such as degree, closeness, betweenness and centrality) of the ceRNAs can be output.

# Usage

```
ceRNA.Net(data, net_direct = TRUE, vertex_size = 20, v.label = TRUE,
    node_shape = "circle",n_color = "orange",E_weight = TRUE, ity = 1,
    label_cex = 2, label_color = "black",edge_color = "gray",
    n_frame.color = "gray")
```

# Arguments

data	A matrix of ceRNA interaction pairs identified by statistical identification meth- ods selected by the user .
net_direct	A logical variable specifies a directed or undirected network.default (net_direct = TRUE).
vertex_size	a numeric vector to adjust the node size, default (vertex_size = 20).
v.label	Whether to display the label of the node.default (v.label = TRUE).
node_shape	A character vector is used to adjust the shape of the node, The selectable shape parameters are "circle", "square", "csquare", "rectangle", "crectangle", "vrectangle", "pie" "sphere", "none".default (node_shape = "circle").
n_color	The character vector used to define the fill color of the node.
E_weight	A logical vector represents whether the network is a weighted network, default ( $E_weight = TRUE$ ).
ity	A numeric vector defines whether the edge is a solid line or a dotted line, the possible values of the vector are $c(1, 2)$ , default (ity = 1).
label_cex	Specify the size of the node label font.
label_color	Specify the label color of the node.
edge_color	Specify the color of the network side.
n_frame.color	The character vector used to define the border color of the node.

### Details

This function calls the igraph package. For specific parameter settings, please refer to the igrap help documentation. Note:All the arguments without default value must be assigned.

## Value

The output includes two parts: the network diagram of ceRNA interaction and the topology attribute information of the network.

Network topology attributes include 5 types of information:

- degree degree refers to the number of edges in the network directly connected to the node
- closeness An indicator that describes the average distance of a node to all other nodes in the network.
- betweenness The proportion of this node that appears in the shortest path between other nodes.
- cluster coefficient Representing the dense connection nature between some nodes in the network
- Eigenvector centrality Representing the characteristic vector centrality of the network.

#### Examples

ceRNA.surv

survival analysis of ceRNA ternary relationship pairs

# Description

It is used to predict the survival of ternary relationship pairs and to support the survival prognosis of training sets and test sets.

#### Usage

```
ceRNA.surv(ceRNA, exp.sur, train = NULL, test = NULL, index)
```

# ceRNA.surv

#### Arguments

ceRNA	a dataframe that the ceRNA relationship is the data, and the prediction result data obtained according to the ceRNA prediction algorithm.Such as ceRNA.cmi prediction result file.
exp.sur	dataframe specifying expression and survival information. Its rownames are sample names. Its colnames are names in triplets and survival information (see example data in details).
train	a character string vector specifying train sample names.
test	a character string vector specifying test sample names.
index	a numeric vector (default 1) representing rowindex of triplets analyed.

# Value

A list of identified miRNA sponge interactions containing following components:

- targetce represented target names, respectively.
- anotherce names of modulators that another target(modulators) constitutes a ceRNA interaction relation.
- coef\_targetce the *coxph* coefficient of targetce;
- p\_targetce the *coxph* significance of targetce;
- coef\_anotherce the *coxph* coefficient of anotherce;
- p\_anotherce the *coxph* significance of anotherce;
- N\_low The genes were ranked according to expression, and the number of samples expressed in the bottom 25
- N\_high The genes were ranked according to expression, and the number of samples expressed in the top 25
- HR Risk score of ceRNA ternary pair.
- p Survival significance of ceRNA ternary pairs.

# Examples

```
##---- Should be DIRECTLY executable !! ----
##-- ==> Define data, use random,
##--or do help(data=index) for the standard data sets.
## The function is currently defined as
ceRNA.surv(ceRNA=dataset[["Pre.ceRNA"]],exp.sur=dataset[["exp.sur"]],train=NULL,test=NULL,index=5)
```

dataset

#### Description

This object contains data for examples.

# Usage

data(dataset)

#### Format

A list with 4 variables:

- **miRtar** MiRNA-target regulation, assembled genome-wide miRNA-gene regulation by TargetScan, It includes 44 rows standing for miRNA-gene regulation pairs in glioblastoma and 2 columns (the first is dataframe header).
- **geneexp** a dataframe representing target regulations expression ,all of which are gene expression value, in glioblastoma. It includes 6 rows standing for 6 gene targets and 541 columns standing for 541 samples. Its rownames are gene symbols.
- miRexp a dataframe representing miRNA expression profile in glioblastoma. It includes 57 rows standing for 57 miRNA and 541 columns standing for 541 samples. Its rownames are miRNA symbols.
- **Pre.ceRNA** a data frame representing the ceRNA identified in the pre-experiment using the method provided by the software to draw a network map.

mres a data frame representing the MRE.

- GOterms a list representing that goterm of interest and all gene sets in the term.
- background a character representing a gene set in which GOterm annotated genes must be.
- **exp.sur** a data frame representing specifying expression and survival information. Its rownames are sample names. Its colnames are names in triplets and survival information
- train a data frame representing specifying train sample names
- test a data frame representing specifying test sample names.

#### Details

All epression data is from a study about glioblastoma. The miRNA-gene regulation information is from TargetScan.

#### Source

Network, Atlas T C G. et al.

# surv.plot

# References

Network, Atlas T C G . Corrigendum: Comprehensive genomic characterization defines human glioblastoma genes and core pathways[J]. Nature, 2013, 494(7438):506-506.

# Examples

```
data(dataset)
## maybe str(dataset) ; ...
```

surv.plot

Draw survival curve

# Description

It is used to draw the survival curve of ternary relationship pairs and to support the survival prognosis of training sets and test sets.

# Usage

surv.plot(ceRNA, exp.sur, train = NULL, test = NULL, index)

# Arguments

ceRNA	a dataframe that the ceRNA relationship is the data, and the prediction result data obtained according to the ceRNA prediction algorithm.Such as ceRNA.cmi prediction result file.
exp.sur	dataframe specifying expression and survival information. Its rownames are sample names. Its colnames are names in triplets and survival information (see example data in details).
train	a character string vector specifying train sample names.
test	a character string vector specifying test sample names.
index	a numeric vector (default 1) representing rowindex of triplets analyed.

# Value

Survival curve of user-selected ceRNA ternary pairs.

# Examples

```
##---- Should be DIRECTLY executable !! ----
##-- ==> Define data, use random,
##--or do help(data=index) for the standard data sets.
## The function is currently defined as
```

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