# miRsponge: an R/Bioconductor package for the identification and analysis of miRNA sponge interaction networks and modules

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# Abstract

### Background

A microRNA (miRNA) sponge is an RNA molecule with multiple tandem miRNA response elements that can sequester miRNAs from their target mRNAs. Despite growing appreciation of the importance of miRNA sponges, our knowledge of their complex functions remains limited. Moreover, there is still a lack of miRNA sponge research tools that help researchers to quickly compare their proposed methods with other methods, apply existing methods to new datasets, or select appropriate methods for assisting in subsequent experimental design.

### Results

To fill the gap, we present an R/Bioconductor package, *miRsponge*, for simplifying the procedure of identifying and analyzing miRNA sponge interaction networks and modules. It provides seven popular methods and an integrative method to identify miRNA sponge interactions. Moreover, it supports the validation of miRNA sponge interactions and the identification of miRNA sponge modules, as well as functional enrichment and survival analysis of miRNA sponge modules.

### Conclusions

This package enables researchers to quickly evaluate their new methods, apply existing methods to new datasets, and consequently speed up miRNA sponge research.



### Keywords

miRNA – ceRNA – miRNA sponge – miRNA sponge interaction networks – miRNA sponge modules – human breast invasive carcinoma


# Background

MicroRNAs (miRNAs) are small non-coding RNAs with ~22 nucleotides. They usually induce repression or translational inhabitation of target genes through partial

complementarities with miRNA response elements (MREs) of the target RNA transcripts [1]. Previous studies [2, 3] have shown that miRNAs are involved in a broad range of biological processes, such as cell cycle control, cell apoptosis, cell differentiation and a diverse range of human cancers.

The competing endogenous RNA (ceRNA) hypothesis [4] considers that all types of coding and non-coding RNA transcripts may crosstalk with each other by sharing MREs. By competing with each other, those ceRNAs (also known as miRNA sponges or decoys), including long non-coding RNAs (lncRNAs), pseudogenes, mRNAs and circular RNAs (circRNAs), can release parental target mRNAs from miRNAs' control [5]. The ceRNA hypothesis challenges the traditional knowledge that coding RNAs only act as targets of miRNAs, and it provides a starting point for the investigation of the biological functions and mechanisms of miRNA sponges.

Recently, several lncRNAs, pseudogenes, circRNAs and mRNAs acting as miRNA sponges have been experimentally identified and confirmed, together with their important biological functions. For example, linc-MD1, a muscle-specific lncRNA, activates muscle-specific gene expression by regulating the expression of MAML1 and MEF2C via sponging miR-133 and miR-135 [6]. PTENP1, a pseudogene of the PTEN tumor suppression gene, can act as a sponge of PTEN-targeting miRNAs to play a growth-suppressive role [7]. As for circRNAs, CDR1as/ciRS-7 is encoded in the genome antisense to the human CDR1 (gene) locus, and affects the activity of miR-7 target genes by sponging miR-7 [8, 9]. Tay *et al.* [10] have validated two mRNAs (CNOT6L and VAPA) as miRNA sponges that regulate tumor suppressor gene PTEN, antagonise PI3K/AKT signalling, and show concordant expression patterns and copy number loss with PTEN in human cancers.

Although an increasing number of miRNA sponges have been discovered, the wet lab experiment approach for finding them is very time consuming and involves high cost. Thus computational methods [10-18] have been proposed to study miRNA sponges. For the identification of miRNA sponge interactions, the two principles, significant sharing of miRNAs at sequence level and positively correlated at expression level, are commonly used in several computational methods. It has been shown that there is a great potential to reveal the biological mechanisms in cancer by studying miRNA sponge networks and modules [5, 11, 19], and the computational methods for identifying miRNA sponge networks and modules can help broaden our study of miRNA sponges and generate hypotheses for wet lab experiments.

However, to date, there are still no software tools available for systematically identifying and analyzing miRNA sponge interaction networks and modules. Specifically, it is still difficult for researchers to choose suitable computational methods to identify miRNA sponge interactions or modules in their own data, or compare the performance of their newly developed methods with existing methods. Moreover, it is time consuming for both bioinformaticians and biologists to obtain existing algorithms for their studies as there is not a tool which contains a comprehensive collection of the algorithms.

To fill this gap, we have developed an R/Bioconductor package, *miRsponge*, to provide a pipeline for the identification and analysis of miRNA sponge interaction networks and modules. To the best of our knowledge, *miRsponge* is the first R/Bioconductor package for the study of miRNA sponge interaction networks and modules.

# Methods

### Pipeline of miRsponge

The *miRsponge* package is written in R. The main pipeline of *miRsponge* is given in Figure 1, which can be separated into three components for the identification and analysis of miRNA sponge interaction networks and modules:

- Identification of miRNA sponge interactions
- Identification of miRNA sponge modules
- Validation and analysis

In the following subsections, we describe the three components of the pipeline in detail.

### Identification of miRNA sponge interactions

To identify miRNA sponge interactions, *miRsponge* provides the *spongeMethod* function which has implemented seven popular methods: *miRHomology* [12, 13], *pc* [14, 15], *sppc* [16], *hermes* [17], *ppc* [11], *muTaME* [10], and *cernia* [18]. In the following, we describe each of the implemented methods in detail.

(1) The *miRHomology* method

The *miRHomology* method identifies miRNA sponge interactions based on the homology of sharing miRNAs. Firstly, the *miRHomology* method generates all possible candidate RNA pairs that share a set of miRNAs based on putative miRNA-target interactions. Then, for each candidate RNA pair ($RNA_i$ and $RNA_j$), the hypergeometric test is used to evaluate the significance of the shared miRNAs by these two RNAs. The significance *p*-value is calculated as follows:

$$p = 1 - \sum_{x=0}^{n-1} \frac{\binom{K}{x}\binom{N-K}{M-x}}{\binom{N}{M}} \quad (1)$$

Here $N$ denotes the number of all miRNAs of interest, $K$ is the number of miRNAs interacting with $RNA_i$, $M$ is the number of miRNAs interacting with $RNA_j$, and $n$ is the number of the shared miRNAs by $RNA_i$ and $RNA_j$. The RNA-RNA pairs with significant sharing of miRNAs (e.g. $p$-value < 0.05) are regarded as miRNA sponge interactions.

(2) The *pc* method

Pearson correlation [20] is a classical method to evaluate the association between a pair of variables. The *pc* method considers gene expression data based on the above two steps of *miRHomology* method. For the identification of miRNA sponge interactions, the correlations between each RNA pair with significant sharing of miRNAs are calculated. The RNA pairs with significant positive correlations (e.g. $p$-value < 0.05) are regarded as miRNA sponge interactions.

(3) The *sppc* method

Different from the *pc* method, the *sppc* method considers both miRNA and mRNA expression data when inferring miRNA sponge interactions. The *sensitivity correlation* (*SC*) between each RNA pair with significant sharing of miRNAs is defined as follows:

$$SC = \rho_{ij} - \rho_{ij|n} \quad (2)$$

In the formula, $\rho_{ij}$ is the Pearson correlation between each candidate RNA pair ($RNA_i$ and $RNA_j$), and $\rho_{ij|n}$ denotes the partial Pearson correlation between $RNA_i$ and $RNA_j$, with the effect of the $n$ shared miRNAs by $RNA_i$ and $RNA_j$ removed. A candidate RNA pair with high value of *SC* is more likely to be a miRNA sponge interaction.

(4) The *hermes* method

As Pearson correlation method only captures linear associations between variables, it cannot handle the problem of non-linear relationships well. For non-linear associations between miRNA sponges, the *hermes* method predicts miRNA sponge interactions via evidence of the sponges competing for miRNA regulation based on mutual information (MI) and conditional mutual information (CMI). For each ($RNA_i$, $miR_k$, $RNA_j$) triplet, the *hermes* method calculates the statistical significance of the difference between the CMI and MI, i.e. $\Delta I = I[miR_k; RNA_i \mid RNA_j] - I[miR_k; RNA_i]$ from matched miRNA and gene expression data using different CMI estimators [21]. The RNA pairs with statistical significance of $\Delta I$ (e.g. $p$-value < 0.05) are regarded as miRNA sponge interactions.

(5) The *ppc* method

The *ppc* method is a variant of the *hermes* method. For the linear associations between miRNA sponges, it identifies miRNA sponge interactions via evidence the sponges competing for miRNA regulation based on Pearson correlation and partial Pearson correlation. For each ($RNA_i$, $miR_k$, $RNA_j$) triplet, the *ppc* method calculates the statistical significance of $\Delta C = C[miR_k; RNA_i \mid RNA_j] - C[miR_k; RNA_i]$ from matched miRNA and gene expression data, where $C$ stands for Pearson correlation. We regard the RNA pairs with statistical significance of $\Delta C$ (e.g. $p$-value < 0.05) as miRNA sponge interactions.

(6) The *muTaME* method

The *muTaME* method is implemented based on the following four scores:

- The fraction of common miRNAs
- The density of the MREs for all shared miRNAs

- The distribution of MREs of the putative RNA-RNA pairs
- The proportion between the overall number of MREs for a putative miRNA sponge compared with the number of miRNAs that yield these MREs

By adding the logarithm of the four scores together, we obtain the combined score for a candidate RNA pair ($RNA_i$ and $RNA_j$). To compare the strength of the pairs, we normalize their combined scores to obtain normalized scores in the range of [0 1], and the RNA pairs ranked high according to their scores (e.g. top 10%) are regarded as miRNA sponge interactions.

(7) The *cernia* method

The *cernia* method is an updated version of the *muTaME* method. It is implemented based on the following seven scores:

- The fraction of common miRNAs
- The density of the MREs for all shared miRNAs
- The distribution of MREs of the putative RNA-RNA pairs
- The proportion between the overall number of MREs for a putative miRNA sponge compared with the number of miRNAs that yield these MREs
- The density of the hybridization energies related to MREs for all shared miRNAs
- The DT-Hybrid recommendation score
- The Pearson correlation between putative RNA-RNA pair expression data

Similarly, the method obtains a combined score by adding the logarithm of these seven scores, then normalizes the combined scores to be in the range of [0 1]. The

RNA pairs with high scores (e.g. top 10%) are regarded as miRNA sponge interactions.

(8) The integrative method

The above seven methods each have its own merit due to different evaluating indicators. Thus, *miRsponge* also provides the *integrateMethod* function to obtain high-confidence miRNA sponge interactions from the predictions made by different methods using majority voting. Specifically, for the predicted miRNA sponge interactions made by the above seven computational methods, we only retain those miRNA sponge interactions that are predicted by at least *k* (e.g. 3) computational methods. Certainly, users can change the setting to use more tools in the voting.

To help users further understand the common and different characteristics of eight miRNA sponge interactions identification methods, we have made a summary of them in Table 1. Depending (or not) on gene expression data for individual method, the interactions can be divided into two types: dynamic and static. The *integrateMethod* approach contains both dynamic and static interactions, thus the type of interactions is hybrid. According to problem of linear or non-linear relationships for individual method, the type of interactions can also be divided into two types (linear and non-linear). Since the *integrateMethod* approach contains both linear and non-linear interactions, the type of interactions is also hybrid. According to the specific needs, users can select a reasonable method for their own. Moreover, regarding choosing the method to achieve the best results and performance metrics, for the same input data, the best method for the identification of miRNA sponge interactions is the one that identifies the largest number of experimentally validated miRNA sponge interactions.

### Identification of miRNA sponge modules

Since modularity is an important property of many biological networks, the discovery and analysis of modules in biological networks have attracted much attention within the bioinformatics community. To understand the module-level properties of miRNA sponges, we implement the *netModule* function to identify miRNA sponge modules from miRNA sponge interaction networks.

Users can select the FN [22], MCL [23], LINKCOMM [24] or MCODE [25] method for module identification. FN is a fast hierarchical agglomeration algorithm, and it is implemented by greedily optimizing the modularity for detecting community structures [22]. MCL relies on the Markov cluster algorithm, and identifies modules in biological networks by a mathematical bootstrapping procedure. The procedure simulates random walks within a biological network by the alternation of two operators called *expansion* and *inflation* [23]. LINKCOMM is a module identification method from the *linkcomm* R package [24]. It clusters the links between nodes rather than clustering nodes to identify modules. The identified modules are allowed to have same nodes, consequently uncovering the overlapping and dense community of a biological network. MCODE identifies densely connected modules based on vertex weighting (local neighbourhood density) and outward traversal (from a locally dense seed node to isolate the dense regions) [25].

For the identification of miRNA sponge modules, users can use the number of significantly enriched terms associated with miRNA sponge modules or the number of significant miRNA sponge modules for distinguishing the high and the low risk tumor samples to select the best module identification method, which is the one that identifies the largest number of significantly enriched terms associated with miRNA sponge modules or the largest number of significant miRNA sponge modules for distinguishing the high and the low risk tumor samples.

### Validation and analysis

Since the ground truth of miRNA sponge interactions is still limited, it is hard to validate predicted miRNA sponge interactions generated by computational methods. In *miRsponge*, we provide the *spongeValidate* function to validate predicted miRNA sponge interactions by using curated miRNA sponge interactions as a source. As for the predicted miRNA sponge interactions that are not included in the source for validation, users need further wet lab experiments to know if the predictions are correct or not. The default ground truth of miRNA sponge interactions is from the miRSponge database [26], and we will continually update the ground truth for accurately evaluating and comparing different methods. Users can also use their own ground truth or wet experiments to validate predicted results. It is noted that miRSponge with the capital *S* in the name is a database of literature-curated miRNA sponge interactions by Wang *et al*. [26], and the name of our R package *miRsponge* contains the lower case *s*.

Furthermore, to understand potential diseases, biological processes and pathways associated with miRNA sponge modules, we provide two functions, *moduleDEA* and *moduleFEA* for functional enrichment analysis. Firstly, we implement the *moduleDEA* function to make disease enrichment analysis of miRNA sponge modules. The disease databases used include Disease Ontology database (DO, http://disease-ontology.org/), DisGeNET database (DGN, http://www.disgenet.org/) and Network of Cancer Genes database (NCG, http://ncg.kcl.ac.uk/). Moreover, the *moduleFEA* function is implemented to conduct functional GO, KEGG and Reactome enrichment analysis of miRNA sponge modules. The ontology databases used are Gene Ontology database (GO, http://www.geneontology.org/), Kyoto Encyclopedia of Genes and Genomes Pathway Database (KEGG, http://www.genome.jp/kegg/), and Reactome Pathway Database (Reactome, http://reactome.org/).

Since the survival analysis can indicate whether the miRNA sponges in the discovered miRNA sponge modules are good predictors of the metastasis risks of cancer patients or not, it can provide us the clue that the miRNA sponges may be associated with and potentially contributing to the metastasis or survival of cancer patients. In *miRsponge*, we implement the *moduleSurvival* function to perform the survival analysis. A multivariate Cox model is used to calculate the risk score of a sample. All the samples are divided into the high risk and the low risk groups according to their risk scores, and the Log-rank test is used to test for the difference between groups. The Hazard Ratio (HR) between the high and the low risk groups is also calculated.

## Results

The *miRsponge* package is developed for the identification and analysis of miRNA sponge interaction networks and modules. In this section, we show the usages of the *miRsponge* package by conducting a case study. For the case study, the dataset used can be obtained from https://github.com/zhangjunpeng411/miRsponge_dataset and the running scripts can be seen in Additional file 1.

In the case study, we only focus on studying mRNA related miRNA sponge interaction networks and modules. It is noted that *miRsponge* can be applied to study the miRNA sponge interaction networks and modules involving other types of RNA (lncRNA, pseudogene, circRNA, etc.). For instance, if we want to consider lncRNAs acting as miRNA sponges, the input miRNA-target interactions and MRE data to *miRsponge* should contain miRNA-lncRNA interactions and the gene expression data should include matched lncRNA expression data.

### Data source

The matched Level 3 IlluminaHiSeq miRNA and mRNA expression data of human breast invasive carcinoma (BRCA) is obtained from Paci *et al*. [16]. The gene-level

expression values are in Fragments Per Kilobase Million (FPKM) units. Paci *et al*. obtained the expression data from The Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/), and restricted the data to 72 individuals where the tumor and normal tissues were from the same BRCA patients. A miRNA or mRNA with missing values in more than 10% of the samples is removed. The remaining missing values are imputed using the *k*-nearest neighbours (KNN) algorithm in the *impute* R package [27]. We use the *limma* R package [28] to infer differentially expressed miRNAs and mRNAs between tumour and normal samples. After the analysis, we identify 161 miRNAs and 5370 mRNAs which are differentially expressed at a significant level (adjusted *p*-value < 1E-04, adjusted by Benjamini & Hochberg method). The survival data of the 72 BRCA tumour samples is obtained from TCGA.

To obtain high-quality candidate miRNA sponge interaction pairs, we use experimentally validated miRNA-target interactions from miRTarBase v7.0 [29] for the case study. We are only interested in the miRNA-target interactions supported by strong experimental evidences (Reporter assay or Western blot) in miRTarBase. Between 161 miRNAs and 5370 mRNAs which are differentially expressed, we obtain 1251 unique miRNA-target interactions from miRTarBase. The MREs information is obtained from Sardina *et al*. [18]. They employ miRanda algorithm [30] to predict binding sites, and MREs have the hybridization energy values no more than 0 are reserved. In total, we have a list of 824828 MREs related to the differentially expressed miRNAs and mRNAs. Since the miRSponge database [26] is far from complete, we also collect validated miRNA sponge interactions from the three manually curated literatures [5, 11, 31]. By integrating the validated miRNA sponge

interactions from both the miRSponge database and the literatures, we have collected a list of 561 unique validated miRNA sponge interactions as the ground truth in total.

### Identification and validation of BRCA-related miRNA sponge interactions

In this section, we use the *miRsponge* package, including the 7 methods introduced in the section *Identification of miRNA sponge interactions* and the integrated method (*integreateMethod*) to identify BRCA-related miRNA sponge interactions. The significant *p*-value cutoff of the shared miRNAs is 0.05. For the *pc* method, the *p*-value cutoff of significant positive correlations is 0.05. For the *sppc* method, the *sensitivity correlation* cutoff is 0.1. As for the *hermes* and *ppc* methods, the statistical significance *p*-value of evaluating $\Delta I$ and $\Delta C$ is 0.05. The number of permutations for both the *hermes* and *ppc* methods is 100. The cutoff of normalized score is 0.5 for the *muTaME* and *cernia* methods. All the *p*-values above are adjusted by the Benjamini & Hochberg method. For the *integrateMethod* method, we only retain the predicted miRNA sponge interactions which are predicted by at least 3 out of the 7 component methods.

As shown in Figure 2, the numbers of predicted miRNA sponge interactions by the 7 individual methods are different. The number of predicted miRNA sponge interactions shared by the 7 methods is 5. Specifically, the *miRHomology* method independently predicted 44 miRNA sponge interactions which are not predicted by any of the other methods. The reason is that the *miRHomology* method only uses a commonly used constraint (significant sharing of common miRNAs) to identify miRNA sponge interactions. By using the *integrateMethod* method, we obtain a list of 87 high-confidence miRNA sponge interactions. The detailed information of predicted miRNA sponge interactions by the methods can be seen in Additional file 2.

To investigate the overlap of any 2 of 7 individual methods, we further use the Venn diagrams to show pair-wise comparison of overlapping results for the 7 individual methods. The detailed information of pair-wise comparison results can be seen in Additional file 3.

Furthermore, we use the ground truth to validate the predicted miRNA sponge interactions. As a result, for all the methods only 1 miRNA sponge interaction, (PTEN:ZEB1, where ":" denotes competing relationship), is validated by the ground truth. In terms of the number of experimentally validated miRNA sponge interactions, these methods perform the same. However, in terms of the percentage of experimentally validated miRNA sponge interactions, the *sppc* method performs the best.

**Module identification from BRCA-related miRNA sponge interaction network**

Since the *integrateMethod* method integrates the results of 7 individual methods (*miRHomology*, *pc*, *sppc*, *hermes*, *ppc*, *muTaME* and *cernia*) to infer high-confidence miRNA sponge interaction network, in this section, we focus on module identification from BRCA-related miRNA sponge interaction network discovered by this integrated method. As shown in Table 2, the four module identification methods (FN, MCL, LINKCOMM and MCODE) obtain different results of the identified miRNA sponge modules. As LINKCOMM allows overlapping nodes between modules, it identified the largest number of miRNA sponge modules.

**Enrichment and survival analysis of BRCA-related miRNA sponge modules**

In this section, we conduct enrichment and survival analysis of the identified BRCA-related miRNA sponge modules described in the previous section. Users can compare the performance of different module identification methods by using the number of significantly enriched terms and significant miRNA sponge modules. Here, if a term

(DO, DGN, NCG, GO, KEGG or Reactome term) is enriched in a miRNA sponge module with adjusted *p*-value < 0.05 (adjusted by Benjamini & Hochberg method), the term is a significantly enriched term. If a miRNA sponge module can distinguish the high and the low risk tumor samples (Log-rank *p*-value < 0.05, HR > 1.5), the miRNA sponge module is regarded as a significant miRNA sponge module.

The overall number of significantly enriched terms can be seen in Figure 3. Overall, the identified modules by the LINKCOMM method have the largest number of significantly enriched DGN, GO and Reactome terms. The identified modules by MCODE have the largest number of significantly enriched DO and NCG terms. The modules identified by the FN method have the largest number of significantly enriched KEGG terms. In terms of the number of significantly enriched terms, LINKCOMM method performs the best. For each method, the number of significantly enriched terms in each module can be seen in Additional file 4. Due to a lack of comprehensive knowledge of enriched terms indirectly and directly associated with BRCA, we use all significantly enriched terms (DO, DGN, NCG, GO, KEGG and Reactome terms) associated with the miRNA sponge modules to compare the performance of each module identification method. It is noted that users can further investigate these significantly enriched terms in the context of BRCA.

As shown in Table 3, the numbers of significant miRNA sponge modules for distinguishing the high and the low risk BRCA samples are 4, 2, 5 and 2 for FN, MCL, LINKCOMM and MCODE methods, respectively. In terms of the number of significant miRNA sponge modules, LINKCOMM method performs the best.

## Discussion

*miRsponge* has been released under the GPL-3.0 License, and is available at http://bioconductor.org/packages/miRsponge/ and https://github.com/zhangjunpeng411/miRsp

onge. The user manual of *miRsponge* provides examples illustrating the use of the fun ctions of *miRsponge*, and can be seen in https://bioconductor.org/packages/release/bioc/vignettes/miRsponge/inst/doc/miRsponge.html.

The putative miRNA-target interactions are crucial for the identification of miRNA sponge interaction. The miRNA-target identification methods, such as miRanda [30], TargetScan [33] and DIANA-microT [34], predict many false positives and the number of miRNA target genes is largely overestimated [35], affecting the accuracy of the candidate miRNA sponge interaction pairs. Therefore, instead of predicted miRNA-target interactions, we use experimentally validated miRNA-target interactions in the case study. The experimentally validated miRNA-target interactions can be used to provide high-quality candidate miRNA sponge interaction pairs. According to the practical situation, users can also use their own predicted miRNA-target interactions to obtain candidate miRNA sponge interaction pairs. Moreover, the number of candidate miRNA sponges is closely associated with the putative miRNA-target interactions used. In the case study, we only use the miRNA-target interactions supported by strong experimental evidences (Reporter assay or Western blot) in miRTarBase. The maximum number of miRNA sponges expected to be identified depends on the number of targets in the putative miRNA-target interactions. However, experimentally validated miRNA-target interactions are far from complete. A possible solution is to integrate multiple experimentally validated datasets, such as miRTarBase [29], TarBase [36] and miRWalk [37], to obtain a more comprehensive list of putative miRNA-target interactions.

## Conclusion

Recently, miRNA sponges have been demonstrated to play important roles in human cancers by modulating the expression of key oncogenes and tumor-suppressor genes.

Since biological experiments generate a large amount of data associated with miRNA sponges, this creates a strong demand for data analysis in statistics computing environments such as R. In this paper, we introduce an R/Bioconductor package that enables R users to use popular computational methods for the identification and analysis of miRNA sponge interaction networks and modules. As the research into miRNA sponges is just emerging, we will continuously include new methods in the package in the future. We believe that *miRsponge* will be a useful tool for the research of miRNA sponges.

## Abbreviations

**miRNA:** microRNA; **MRE:** miRNA response element; **ceRNA:** Competing endogenous RNA; **lncRNA:** Long non-coding RNA; **circRNA:** Circular RNA; **SC:** Sensitivity correlation; **MI:** Mutual information; **CMI:** Conditional mutual information; **DO:** Disease ontology; **NCG:** Network of Cancer Genes; **GO:** Gene Ontology; **KEGG:** Kyoto Encyclopedia of Genes and Genomes Pathway; **HR:** Hazard Ratio; **BRCA:** Breast invasive carcinoma; **FPKM:** Fragments Per Kilobase Million; **TCGA:** The Cancer Genome Atlas; **KNN:** *k*-nearest neighbours

## Declarations


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### *Availability of data and materials*

The datasets in the current study are available at https://github.com/zhangjunpeng411/miRsponge_dataset.

### *Authors' contributions*

JZ and TDL conceived the idea of this work. LL, TX and JL refined the idea. JZ designed and performed the experiments. TX, YX and CZ participated in the design of the study and performed the statistical analysis. JZ, TDL, LL, TX and JL drafted the manuscript. All authors revised the manuscript. All authors read and approved the final manuscript.

### *Competing interests*

The authors declare that they have no competing interests.

### *Consent for publication*

Not applicable.

### *Ethics approval and consent to participate*

Not applicable.

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# Figures

**Figure 1 - Pipeline of the miRsponge package**

The pipeline mainly contains three components: Identification of miRNA sponge interactions, Identification of miRNA sponge modules, and Validation and analysis. For the identification of miRNA sponge interactions, out of the eight implemented methods, seven (*miRHomology*, *pc*, *sppc*, *ppc*, *hermes*, *muTaME* and *cernia*) are stand-alone methods and one (*integrateMethod*) is an ensemble method which integrates the prediction results of the component methods. To understand the module-level properties of miRNA sponges, four module identification methods (FN, MCL, LINKCOMM and MCODE) are provided to identify miRNA sponge modules based on the identified miRNA sponge interaction networks. For the validation of miRNA sponge interactions, the ground truth data is used to validate predicted miRNA sponge interactions. Furthermore, enrichment analysis is performed to identify potential diseases, biological processes and pathways associated with miRNA sponge modules. Survival analysis is also performed to identify significant miRNA sponge modules which can distinguish the high and low risk tumor samples. Users can prepare their own datasets as the input to *miRsponge*.

**Figure 2 - UpSet plot [32] to show the intersections between predicted miRNA sponge interactions by the 7 built-in individual methods.**

The intersection size between different methods represents exclusive intersections, i.e. the intersection set not in a subset of any other intersection set.

**Figure 3 - Overall number of significantly enriched terms in BRCA-related miRNA sponge modules using FN, MCL, LINKCOMM and MCODE.**

The significantly enriched terms include DO, DGN, NCG, GO, KEGG and Reactome terms.

# Tables

**Table 1 - Summary of the eight methods for identifying miRNA sponge interactions.**

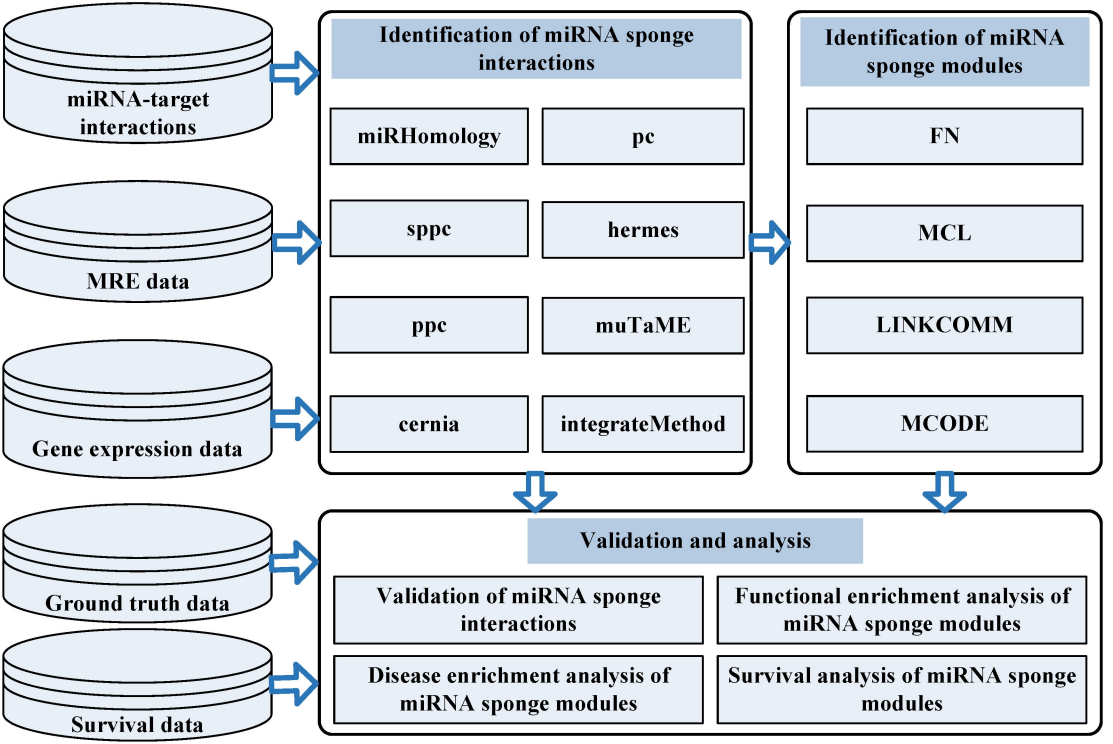
**Table 2 - BRCA-related miRNA sponge modules using FN, MCL, LINKCOMM and MCODE methods.**

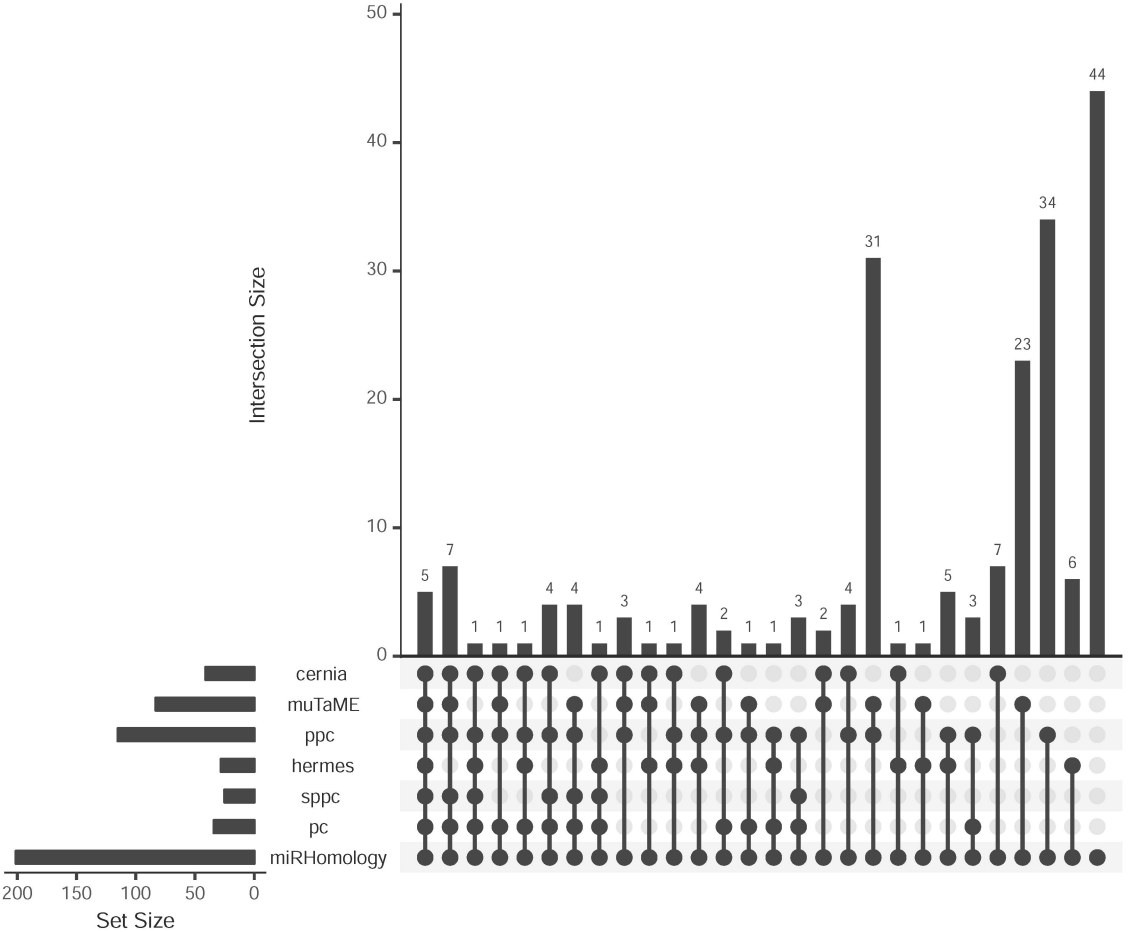
**Table 3 - Survival analysis of BRCA-related miRNA sponge modules using FN, MCL, LINKCOMM and MCODE.**

HRlow95 and HRup95 denote the lower and upper of 95% confidence interval of HR, respectively. The identified significant miRNA sponge modules can distinguish the high and the low risk BRCA samples.

## Additional files

**Additional file 1 – The used running R scripts for reproducing the results of the case study.**
**Additional file 2 – The predicted miRNA sponge interactions by the 8 built-in methods.**
**Additional file 3 – Pair-wise comparison results of the 7 built-in individual method.**
**Additional file 4 – The number of significantly enriched terms in each BRCA-related miRNA sponge module using FN, MCL, LINKCOMM and MCODE.**
The significantly enriched terms contain DO, DGN, NCG, GO, KEGG and Reactome terms.


miRNA-target interactions
MRE data
Gene expression data
Ground truth data
Survival data
Identification of miRNA sponge interactions
miRHomology
pc
sppc
hermes
ppc
muTaME
cernia
integrateMethod
Identification of miRNA sponge modules
FN
MCL
LINKCOMM
MCODE
Validation and analysis
Validation of miRNA sponge interactions
Functional enrichment analysis of miRNA sponge modules
Disease enrichment analysis of miRNA sponge modules
Survival analysis of miRNA sponge modules


Intersection Size
50
40
30
20
10
0
5
7
1
1
1
4
4
1
3
1
1
4
2
1
1
3
2
4
31
1
1
5
3
7
23
34
6
44
cernia
muTaME
ppc
hermes
sppc
pc
miRHomology
200
150
100
50
0
Set Size

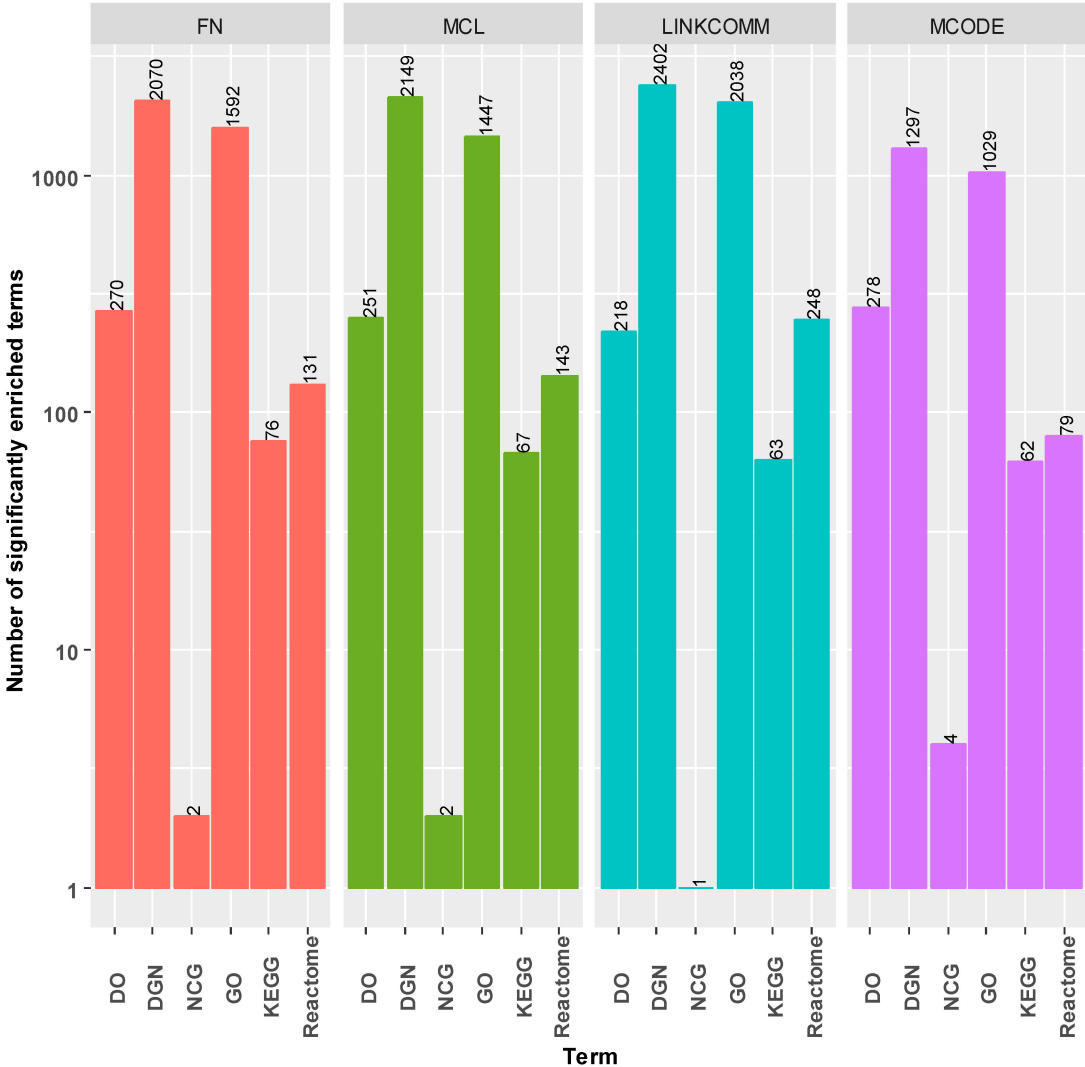

FN
MCL
LINKCOMM
MCODE
Number of significantly enriched terms
1000
100
10
1
270
2070
2
1592
76
131
251
2149
2
1447
67
143
218
2402
1
2038
63
248
278
1297
4
1029
62
79
DO
DGN
NCG
GO
KEGG
Reactome
Term

**Table 1 Summary of the eight methods for identifying miRNA sponge interactions**

| Methods | Input | Type of interactions | Advantages/disadvantages |
|---|---|---|---|
| miRHomology | miRNA-target interactions | static | • the number of miRNA sponges is largely overestimated<br>• ignore gene expression data and MREs information<br>• simple and fast |
| pc | miRNA-target interactions, gene expression data | dynamic, linear | • ignore non-linear interactions<br>• ignore miRNA expression data and MREs information<br>• simple and fast |
| sppc | miRNA-target interactions, gene expression data | dynamic, linear | • ignore non-linear interactions<br>• ignore MREs information<br>• employ sensitivity correlation to evaluate the influence of miRNAs |
| hermes | miRNA-target interactions, gene expression data | dynamic, non-linear | • ignore MREs information<br>• time consuming<br>• capture non-linear interactions by calculating the statistical significance of $\Delta I$ |
| ppc | miRNA-target interactions, gene expression data | dynamic, linear | • ignore non-linear interactions<br>• ignore MREs information<br>• time consuming<br>• capture linear interactions by calculating the statistical significance of $\Delta C$ |
| muTaME | miRNA-target interactions, MREs | static | • ignore gene expression data<br>• consider MREs information |
| cernia | miRNA-target interactions, gene expression data, MREs | dynamic, linear | • ignore non-linear interactions<br>• ignore miRNA expression data<br>• consider MREs information |
| integrateMethod | miRNA sponge interaction networks | hybrid | • contain dynamic and static interactions<br>• include linear and non-linear interactions<br>• high-confidence but time consuming |

**Table 2 BRCA-related miRNA sponge modules using FN, MCL, LINKCOMM and MCODE methods**

| Module methods | Module ID | #miRNA sponges | miRNA sponges |
|---|---|---|---|
| FN | 1 | 14 | *MYB, EIF4E, E2F3, CDC25A, DNMT3A, VEGFA, BCL2, CCNE1, ETS1, DNMT3B, BIRC5, KDR, FGF2, CCND3* |
| | 2 | 12 | *NOTCH1, ZEB1, ZEB2, ZFPM2, TCF7L1, ELMO2, KLHL20, KLF11, CCNE2, DLC1, WASF3, WDR37* |
| | 3 | 7 | *NFIA, FOXO1, BDNF, RECK, CREB1, SNAI2, RUNX2* |
| | 4 | 5 | *PTEN, KLF4, TIMP2, PPARA, BCL2L11* |
| | 5 | 4 | *CDK6, MITF, KRAS, CCND2* |
| | 6 | 3 | *SMAD4, TP53INP1, CASP3* |
| MCL | 1 | 5 | *PTEN, KLF4, TIMP2, PPARA, BCL2L11* |
| | 2 | 12 | *NOTCH1, ZEB1, ZEB2, ZFPM2, TCF7L1, ELMO2, KLHL20, KLF11, CCNE2, DLC1, WASF3, WDR37* |
| | 3 | 10 | *EIF4E, E2F3, CDC25A, VEGFA, BCL2, CCNE1, ETS1, BIRC5, FGF2, CCND3* |
| | 4 | 4 | *CDK6, MITF, KRAS, CCND2* |
| | 5 | 3 | *SMAD4, TP53INP1, CASP3* |
| | 6 | 5 | *NFIA, FOXO1, BDNF, RECK, SNAI2* |
| LINKCOMM | 1 | 4 | *MYB, ZEB2, ZEB1, DLC1* |
| | 2 | 5 | *ZEB1, ZEB2, ZFPM2, KLF11, WDR37* |
| | 3 | 3 | *FOXO1, RECK, SNAI2* |
| | 4 | 4 | *E2F3, CDC25A, CCNE1, CCND3* |
| | 5 | 7 | *PTEN, ZEB1, ZFPM2, TCF7L1, KLHL20, KLF11, WDR37* |
| | 6 | 3 | *CDC25A, BCL2, CCNE1* |
| | 7 | 5 | *PTEN, TIMP2, KLF4, PPARA, BCL2L11* |
| | 8 | 4 | *EIF4E, E2F3, BIRC5, FGF2* |
| | 9 | 6 | *E2F3, MYB, ZEB2, CCNE2, TCF7L1, KLF11* |
| | 10 | 4 | *NOTCH1, ZEB1, ZEB2, ETS1* |
| | 11 | 3 | *VEGFA, E2F3, ETS1* |
| | 12 | 7 | *ZEB1, ZEB2, ZFPM2, TCF7L1, KLHL20, KLF11, DLC1* |
| MCODE | 1 | 25 | *CDK6, NFIA, EIF4E, DNMT3A, MITF, BDNF, PIM1, KLF4, CREB1, SMAD4, SMAD3, KRAS, CCND2, TIMP2, DNMT3B, TWIST1, BIRC5, KDR, FGF2, PPARA, RUNX2, TP53INP1, CASP3, SMAD2, BCL2L11* |

| | | | |
|---|---|---|---|
| | 2 | 24 | *MYB, PTEN, FOXO1, NOTCH1, ZEB1, ZEB2, ZFPM2, E2F3, CDC25A, TCF7L1, RECK, VEGFA, ELMO2, BCL2, KLHL20, KLF11, CCNE2, CCNE1, ETS1, DLC1, SNAI2, WASF3, WDR37, CCND3* |

**Table 3 Survival analysis of BRCA-related miRNA sponge modules using FN, MCL, LINKCOMM and MCODE**

| Module methods | Module ID | Chi-square | *p*-value | HR | HRlow95 | HRup95 |
|---|---|---|---|---|---|---|
| FN | 1 | 9.96 | 1.60E-03 | 2.75 | 1.41 | 5.38 |
| | 2 | 8.32 | 3.92E-03 | 2.57 | 1.33 | 4.96 |
| | 3 | 4.34 | 3.71E-02 | 1.95 | 1.00 | 3.80 |
| | 5 | 4.72 | 2.98E-02 | 2.00 | 1.04 | 3.87 |
| MCL | 2 | 8.32 | 3.92E-03 | 2.57 | 1.33 | 4.96 |
| | 4 | 4.72 | 2.98E-02 | 2.00 | 1.04 | 3.87 |
| LINKCOMM | 2 | 4.78 | 2.88E-02 | 2.03 | 1.04 | 3.94 |
| | 5 | 14.67 | 1.28E-04 | 3.24 | 1.64 | 6.43 |
| | 9 | 3.97 | 4.62E-02 | 1.94 | 1.01 | 3.73 |
| | 10 | 5.41 | 2.00E-02 | 2.17 | 1.12 | 4.18 |
| | 12 | 9.88 | 1.67E-03 | 2.81 | 1.45 | 5.46 |
| MCODE | 1 | 23.83 | 1.05E-06 | 4.52 | 2.26 | 9.03 |
| | 2 | 23.84 | 1.05E-06 | 4.94 | 2.52 | 9.69 |

HRlow95 and HRup95 denote the lower and upper of 95% confidence interval of HR, respectively. The identified significant miRNA sponge modules can distinguish the high and the low risk BRCA samples.