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Analysis of Circular RNAs Using the Web Tool CircInteractome

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Abstract

Circular RNAs (circRNAs) are generated through nonlinear back splicing, during which the 5' and 3' ends are covalently joined. Consequently, the lack of free ends makes them very stable compared to their counterpart linear RNAs. By selectively interacting with microRNAs and RNA-binding proteins (RBPs), circRNAs have been shown to influence gene expression programs. We designed a web tool, CircInteractome, in order to (1) explore potential interactions of circRNAs with RBPs, (2) design specific divergent primers to detect circRNAs, (3) study tissue- and cell-specific circRNAs, (4) identify gene-specific circRNAs, (5) explore potential miRNAs interacting with circRNAs, and (6) design specific siRNAs to silence circRNAs. Here, we review the CircInteractome tool and explain recent updates to the site. The database is freely accessible at http://circinteractome.nia.nih.gov.

Keywords

RNA-binding proteins; Gene-specific circRNAs; Divergent primer design; Cell- and tissue-specific circRNAs; Transcriptome; CLIP-seq

1 Introduction

The past few decades have yielded an ever-growing list of noncoding RNA species with various functions [1]. Circular RNAs (circRNAs) represent a new class of widely expressed and abundant RNA species in eukaryotes [2]. They are generally formed by "head-to-tail" or "back splicing" of pre-mRNA, whereby the 5′ and 3′ ends are covalently linked to form a closed RNA molecule [3, 4]. Although circRNAs have been known for many years, their functions are not as well characterized as those of other noncoding (nc)RNAs such as microRNAs (miRNAs) and long ncRNAs (lncRNAs). However, recent studies have discovered that circRNAs can alter gene expression by interacting with, and in some cases sequestering, miRNAs, RBPs, and splicing factors, thereby altering their availability to protein-coding mRNAs [5, 6]. It has also been suggested that circRNAs could regulate mRNA transcription and translation by interacting with specific RNAs and proteins [5], and the presence of internal ribosome entry sites (IRESs) on some circRNAs has prompted the suggestion that some circRNAs may be translated [7].

RBPs potently regulate gene expression programs at multiple posttranscriptional levels [8]. Recent developments in the analysis of RNA-protein (RNP) interactions using cross-linking techniques have identified with unprecedented precision the binding sequences of RBPs on target RNAs. MicroRNAs are another major class of factors that control gene expression, generally by associating with target mRNAs that share partial complementarity and suppressing mRNA translation and/or stability [9]. We recently developed a new computational resource named "CircInteractome" [7] which enables the systematic exploration of possible interactions of human circRNAs with RBPs and miRNAs (Fig. 1). In this chapter, we describe in detail this tool and the latest updates we have incorporated into it.

2 Materials

- 1. Desktop with Windows operating system (Mac OSX and Linux are adequate too) and Internet Explorer, Mozilla Firefox, or Google Chrome browsers.
- 2. Microsoft Internet Explorer or Mozilla Firefox or Google Chrome browsers.

3 Methods

3.1 Analysis of Interactions of RBPs with circRNAs (Fig. 2)

CircInteractome uses circRNA IDs described in CircBase (http://www.circbase.org), a public database that was developed to gather unified datasets of circRNA IDs, genomic coordinates, and transcripts [10]. The data provided in CircInteractome are predicted based on sequence matches; accordingly, the impact of potential secondary or tertiary structures on circRNA sequence available for interaction with RBPs cannot be considered systematically. Thus, experimental validation is essential to verify circRNA binding to a predicted RBP.

- **1.** Type http://circinteractome.nia.nih.gov into the browser and click "Enter". The home page briefly explains the use of CircInteractome.
- 2. Click on the "circRNA" tab on the left side.
- **3.** Enter the name of the circRNA (e.g., hsa_circ_0000067) in the specified field and click "circRNA Search".
- 4. The new page will show the following features for the "hsa_circ_0000067":
 - a. Genomic location
 - **b.** Length of the genomic and mature circRNA sequences
 - c. Best matched counterpart mRNA (best transcript)
 - **d.** Tissues or cell lines where this circRNA was detected (Samples)
 - e. Studies reporting this circRNA (Study)
- 5. This webpage also shows the RBPs with possible binding sites on this circRNA. For instance, hsa_circ_0000067 has five binding sites for AGO2 and two binding sites for HuR (ELAVL1).
- **6.** The user can further search for the RBP-binding sites on the circRNA junction sequence and flanking region. For example, HNRNPC has one site in the flanking sequence upstream of hsa_circ_0000067.

3.2 CircRNA Genomic Sequence and Mature circRNA Sequence (Fig. 3)

It can be challenging to find the mature circRNA sequence from the UCSC browser mirror (http://genome.mdc-berlin.de). With CircInteractome, the user can easily access genomic and mature circRNA sequences following a few simple steps. The circRNAs are usually predicted from unique junction sequences. The sequence of mature circRNA is predicted from the exon sequences present in that specific genomic location. Further multi-exon circRNAs may or may not include all of the exons and introns in the mature circRNA forms, which will need to be verified experimentally.

- 1. Type http://circinteractome.nia.nih.gov in the web browser and click "Enter".
- 2. Click on the "Circular RNA" tab on the left side.
- **3.** Enter the name of the circRNA (e.g., hsa_circ_0000067) in the specified field and click on the "circRNA Search".
- 4. The new webpage shows various features for the "hsa_circ_0000067".
- 5. Click on the "hsa_circ_0000067" next to "GenomicSeq" or "Mature Seq" to find the genomic or mature circRNA sequence, respectively.

3.3 Specific circRNA Gene (Fig. 4)

- 1. Type http://circinteractome.nia.nih.gov in the web browser and click "Enter".
- 2. Click on the "Circular RNA" tab on the left side.
- **3.** Enter the name of the gene, e.g., *GAPDH*, on the specified field and click on the "circRNA Search".
- 4. The new page shows a table containing the name, genomic location, and length of circRNAs generated from the *GAPDH* gene.
- 5. This table also includes the tissues and cell types wherein the circRNAs were detected (below).
- 6. The user can also download the table as an Excel spreadsheet by clicking on the "excel sheet" logo on top of the table.

3.4 Cell Types and Tissues Where circRNAs Are Expressed (Fig. 5)

- 1. Type http://circinteractome.nia.nih.gov in the web browser and click "Enter".
- 2. Click on the "Circular RNA" tab on the left side.
- **3.** Select the name of the tissue or cell type of interest (e.g., HEK293) from the drag-down list and click on the "circRNA Search".
- 4. The new page shows a table containing the name, genomic location, and length of circRNAs expressed in HEK293 cells.
- 5. This table also shows other tissues or cell types where these circRNAs are reported to be expressed along with HEK293 cells (highlighted).

6. The user can also download the table in the form of Excel spreadsheet by clicking on the "excel sheet" logo on top of the table.

3.5 miRNAs Targeting circRNAs (Fig. 6)

Note: The predicted miRNAs may not bind to circRNAs in vivo due to a lack of accessibility of binding site or lack of miRNA expression. As for RBPs, experimental validation is also necessary for microRNAs. Furthermore, miRNAs which interact in vivo but are not predicted by TargetScan search algorithms may be missed.

- 1. Type http://circinteractome.nia.nih.gov into the browser and click "Enter".
- 2. Click on the "miRNA Target Sites" tab on the left side.
- **3.** Enter the name of the circRNA (e.g., hsa_circ_0000094) in the specified field and click on the "miRNA Target Search". CircInteractome uses the TargetScan Perl script to predict the miRNAs which have sequence complementarity with circRNAs [11].
- 4. The new page shows the predicted miRNAs and number of sites in "hsa_circ_0000094".
- **5.** This page also shows base-pairing and details of the miRNA-circRNA interactions.

3.6 CircRNA Divergent Primer Design (Fig. 7)

Note: The divergent primers designed here may detect the circRNA splice variants coming from the same primary transcript. The user may need to sequence the PCR product to find the exact circRNA junction of interest.

- 1. Type http://circinteractome.nia.nih.gov in the web browser and click "Enter".
- 2. Click on the "Divergent Primers" tab on the left side.
- **3.** Enter the name of the circRNA (e.g., hsa_circ_0000094) in the specified field and click on the "Divergent Primers Search".
- **4.** The new page shows the circRNA junction sequence and two options for divergent primer design.
- 5. The user can click on either "Primer3" or "NCBI Primer Design" and the new webpage will design five pairs of primers for the circRNA of interest "hsa_circ_0000094".

3.7 Designing Primers for Linear Counterpart RNA (Fig. 8)

- 1. Type http://circinteractome.nia.nih.gov in the web browser and click "Enter".
- 2. Click on the "Circular RNA" tab on the left side.
- **3.** Enter the name of the circRNA (e.g., hsa_circ_0000094) on the specified field and click on the "circRNA Search".

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- **4.** The new page shows the following features for the "hsa_circ_0000094", e.g., genomic location, length of genomic and mature circRNA sequence, and best matching counterpart mRNA (best transcript).
- 5. Clicking the hyperlink "Primers" next to "Best Transcript" will open a new page for NCBI primer design tool. The user can design primers by clicking on the "Primers" tab.

3.8 siRNAs Targeting circRNAs (Fig. 9)

Note: The siRNAs have at least ten nucleotides base pairing with the linear counterpart mRNA which might work as miRNA/siRNA. The user may try several siRNAs to identify one that specifically knocks down the circRNA without affecting the linear counterpart mRNA.

- 1. Type http://circinteractome.nia.nih.gov in the browser and click "Enter".
- 2. Click on the "siRNA Design" tab on the left side.
- **3.** Enter the name of the circRNA, e.g., hsa_circ_0000094, in the specified field and click on the "siRNA Search".
- 4. The new webpage shows the ten best possible siRNA target sequences for "hsa_circ_0000094".
- 5. The users may purchase the siRNAs from any source. However, links to order siRNAs from IDT or Dharmacon are provided. The user may wish to add two additional nucleotides (dTdT) as 3' DNA overhangs to the siRNA for better silencing effects.

4 Summary

- 1. In summary, CircInteractome facilitates the study of circRNAs, as well as the RBPs and nucleic acids with which circRNAs associate. The information provided by this tool can illuminate possible functions of circRNAs as molecules that (1) sequester RBPs, microRNAs, and/or other interacting molecules to reduce their availability to mRNAs, (2) function as potential support structures for the assembly of multimolecular complexes, (3) may be translated giving rise to peptides and proteins, (4) interact with mRNAs, thereby promoting or repressing their stability or translation, etc.
- 2. CircInteractome facilitates the design of siRNAs for silencing circRNAs and oligomers for detecting (by RT-qPCR analysis) the circRNAs and the parent RNAs from which they arise.
- **3.** CircInteractome will continue to expand to include additional circRNAs, including circRNAs from other species. It will also search against more databases of RBPs, microRNAs, and interacting molecules as they become available.

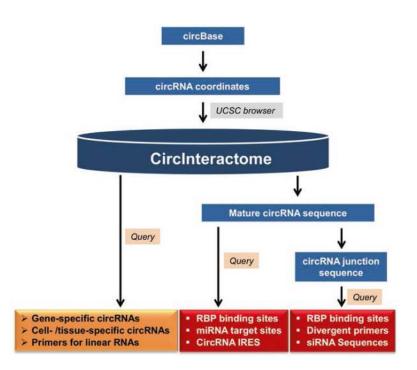
 Collectively, CircInteractome will accelerate our efforts to elucidate the functions of circRNAs in development and disease.

Acknowledgments

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References

- 1. Morris KV, Mattick JS. The rise of regulatory RNA. Nat Rev Genet. 2014; 15:423–437. [PubMed: 24776770]
- Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF, Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. 2013; 19:141–157. [PubMed: 23249747]
- Schindewolf C, Braun S, Domdey H. In vitro generation of a circular exon from a linear pre-mRNA transcript. Nucleic Acids Res. 1996; 24:1260–1266. [PubMed: 8614628]
- 4. Starke S, Jost I, Rossbach O, Schneider T, Schreiner S, Hung LH, Bindereif A. Exon circularization requires canonical splice signals. Cell Rep. 2015; 10:103–111. [PubMed: 25543144]
- Chen LL. The biogenesis and emerging roles of circular RNAs. Nat Rev Mol Cell Biol. 2016; 17:205–211. [PubMed: 26908011]
- Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature. 2013; 495:384–388. [PubMed: 23446346]
- Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K, Gorospe M. CircInteractome: a web tool for exploring circular RNAs and their interacting proteins and microRNAs. RNA Biol. 2016; 13:34–42. [PubMed: 26669964]
- Glisovic T, Bachorik JL, Yong J, Dreyfuss G. RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett. 2008; 582:1977–1986. [PubMed: 18342629]
- Behm-Ansmant I, Rehwinkel J, Izaurralde E. MicroRNAs silence gene expression by repressing protein expression and/or by promoting mRNA decay. Cold Spring Harb Symp Quant Biol. 2006; 71:523–530. [PubMed: 17381335]
- Glazar P, Papavasileiou P, Rajewsky N. circBase: a database for circular RNAs. RNA. 2014; 20:1666–1670. [PubMed: 25234927]
- Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol Cell. 2007; 27:91–105. [PubMed: 17612493]





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Fig. 2.

View of CircInteractome input and output pages. Screenshot of "Circular RNA search" for hsa_circ_0000067, showing RBPs binding to the body and the flanking sequence of this circRNA

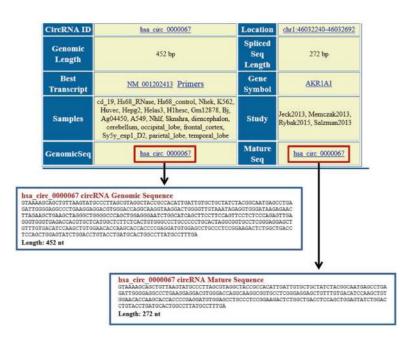


Fig. 3.

Genomic and mature sequences of a given circRNA. Illustrative screenshots from the CircInteractome showing the genomic (left) and mature circRNA (right) sequence for hsa_circ_0000067

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hsa circ 0025177	chr12:6643656-6647536	5 3880	1310	A549, Ag04450, Bj, Gm12878, H1hesc, Helas3, Hepg2, Hmec, Hsmr Huvec, K562, Mcf7, Nhek, Nhlf, Sknshra
hsa circ 0025178	chr12:6643975-6646382	2 2407	466	Ag04450, Bj, Hepg2, Hsmm, Huvec
hsa circ 0025179	chr12:6643975-6646556	5 2581	548	Bj. K562
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hsa circ 0025181	chr12:6643975-6647530	5 3561	1231	A549, Ag04450, Bj, Gm12878, H1hesc, Helas3, Hepg2, Hsmm, Huvec, K562, Mcf7, Nhek, Nhlf, Sknshra
hsa circ 0025182	chr12:6645659-6645759	9 100	100	Gm12878
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hsa circ 0025184	chr12:6645659-6646556	5 897	496	Helas3, Sknshra
hsa circ 0025185	chr12:6645659-6647163	2 1503	909	Ag04450, Bj
hsa circ 0025186	chr12:6645659-6647536	5 1877	1179	A549, Ag04450, Bj, Gm12878, H1hesc, Helas3, Hepg2, Hsmm, Huvec, K562, Mcf7, Nhek, Nhlf, Sknshra
hsa circ 0025187	chr12:6645849-6645956	5 107	107	A549, Ag04450, Gm12878, H1hesc, Mcf7, Nhek
hsa circ 0025188	chr12:6645849-6646176	5 327	198	Gm12878, Hsmm, K562, Nhek
hsa circ 0025189	chr12:6645849-664638.	2 533	314	A549, H1hesc
hsa circ 0025190	chr12:6645849-6646556	5 707	396	H1hesc, Helas3, Nhlf, Sknshra
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Fig. 4.

Gene-specific circRNAs. For a given gene symbol entered, the circRNAs generated from that gene are identified

Excel sheet

download

Circular RNA	Step1: Enter your circRN. your cell line/tissue of inte	A of interest / Gene of interest o	r select
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Fig. 5.

Cell/tissue-specific circRNAs. CircRNAs identified in previous studies in specific cell types or tissues are identified

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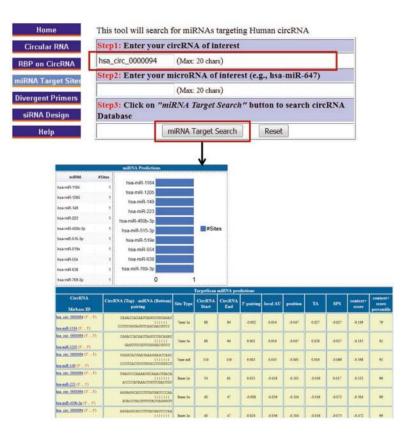


Fig. 6.

Potential miRNA-circRNA interactions. For a given circRNA ID entered (hsa_circ_0000094), the miRNAs potentially targeting hsa_circ_0000094 are identified

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Fig. 7.

Divergent primer design. Screenshots of input and output webpage for divergent primer design for hsa_circ_0000094. Screenshot of the output page showing the junction sequence of circRNA and links of primer design tools (Primer3 and NCBI)

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Fig. 8.

Primer design for counterpart linear RNA. Screenshots of input and output webpage for designing primer for the linear RNA counterpart using NCBI primer design tool

Author Manuscript

Home	This tool will searc	h for siRNA against	Human circRNA June	ction
Circular RNA	Step1: Enter your	circRNA of interes	t (Full Symbol)	
RBP on CircRNA	hsa_circ_0000094	(Max: 20 chars)		
miRNA Target Sites	Step 2: Click on "3		on to generate sikN.	•
Divergent Primers		siRNA Search	Reset	
siRNA Design				
Help				

S.No.	siRNA Target Sequence Please click on siRNA Target Sequence link to run blast search on NCBI server
1	GCCAGGCAGGTATGACAGCTT
2	AGCCAGGCAGGTATGACAGCT
3	AAGGAGCCAGCCAGGCAGGTA
4	GCCAGCCAGGCAGGTATGACA
5	GAGCCAGCCAGGCAGGTATGA
6	AGGAGCCAGCCAGGCAGGTAT
7	AAAGGAGCCAGCCAGGCAGGT
8	AGCCAGCCAGGCAGGTATGAC
9	GGAGCCAGCCAGGCAGGTATG
10	CCAGCCAGGCAGGTATGACAG
	Dharmacon Custom siRNA IDT siRNA Please add "dTdT" 3' DNA overhang

Fig. 9.

CircRNA siRNA design. Screenshot of input and output webpage of CircInteractome siRNA design, including an example of output siRNAs targeting the junction sequence of hsa_circ_0000094