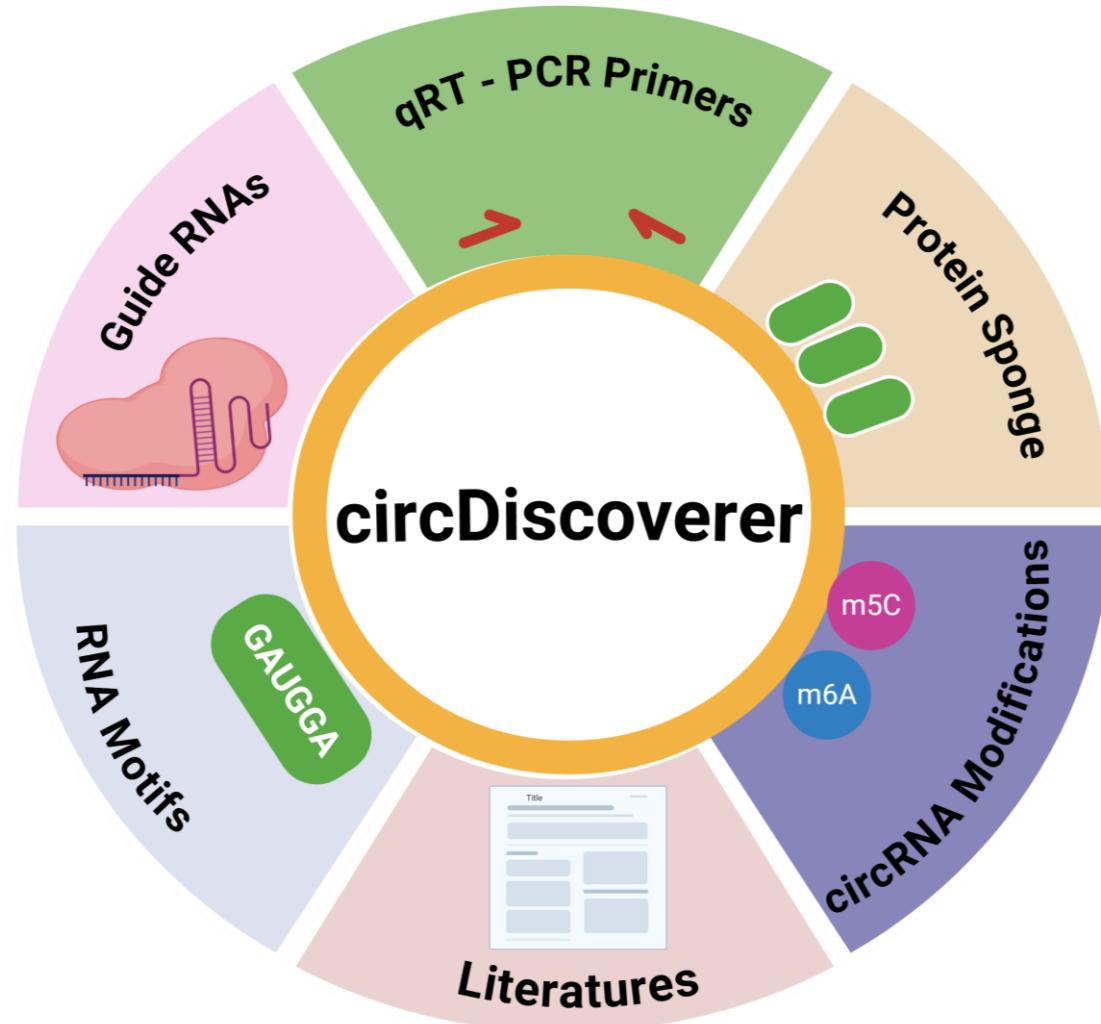


# circDiscoverer Tutorial Page



# Literature Section

Explore the circRNA-Protein Interaction reported in the literatures

Enter the name of protein & get the following details (Page 2 & 3) :

E.g. LIN28B in this case

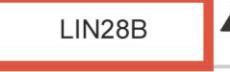
## circDiscoverer

A database for exploring human circRNA–protein interactions

Home   **Literature Section**   Predicted Section   Explore circRNA Section   Help   Download

### Explore Literatures of circRNA–Protein Interaction

circRNAs binding to LIN28B

Select or Type Protein:   

circBase ID	circAtlas ID	Gene	Disease/Physiology	Function	Drugs	Modification	DOI
<a href="#">hsa_circ_0058058</a>	<a href="#">hsa-ATIC_0018</a>	ATIC	Bladder Cancer	Promote	Cisplatin	N/A	<a href="#">DOI</a>

Figure 1

## Details of the Literature Section

- hg19 : The circbase ID of the binding circRNA.
- hg38 : The circAtlas ID of the binding circRNA.
- Gene : The gene from which the circRNA is derived.
- Disease : The disease in which this circRNA-Protein Interaction is reported.
- Function : Function of this circRNA-Protein interaction in the respective disease, whether the circRNA-Protein interaction either promotes or inhibits the respective disease.
- Drugs : The drugs, whose response is affected in the disease by this circRNA-Protein Interaction.
- Modifications : The modifications found in the interacting circRNA.
- DOI of the literature.

# Predicted Section

Get possible circRNA's binding to the proteins

The screenshot shows the circDiscoverer website interface. The top navigation bar includes links for Home, Literature Section, Predicted Section (which is highlighted with a red box), Explore circRNA Section, Help, and Download. Below the navigation is a sub-header: "Get circRNA's Binding to your Protein". A search input field contains the text "FXR1", also highlighted with a red box. The main content area displays a table titled "circRNAs binding to FXR1", which is also highlighted with a red box. The table has columns for circRNA, Sample Count, Sample Score, Type, and Tools. Three rows are listed:

circRNA	Sample Count	Sample Score	Type	Tools
<a href="#">hsa-RMRP_0001</a>	5	1.000	CLIP-Seq	circExplorer2
<a href="#">hsa-SH2B3_0001</a>	3	0.600	CLIP-Seq	CIRI2, circExplorer2
<a href="#">hsa-RMRP_0005</a>	2	0.400	CLIP-Seq; Motif	CIRI2

Figure 2

Clicking on any of these circRNA's, for e.g. hsa-RMRP\_0001, will directly land in the circAtlas database (Page 5)

# circAtlas 3.0 Results Page



## circAtlas 3.0 a gateway to 3 million standardized named vertebrate circular RNAs

Home Data Top30 Conserved Convert Function Network Disease Download Tutorial circSC CIRhub

Search result of hsa-RMRP\_0001

Show 50 entries Search:

Species	circAtlas ID	Uniform ID	Position	Strand	circRNA type	Host gene	Multiple Conservation Score	MCS-detail			Tissue Specific Index
								species	tissue	sample	
human	hsa-RMRP_0001	circ(chr9).87	chr9:35657751 35658018	-	Exonic	ENSG00000269900.3	1.15152	1	17	53	1

Figure 3

## Details of the Predicted Section

- **circRNA** : List of circRNAs binding to the protein of interest.
- **Sample Count** : Its the total number of samples in which a particular circRNA is found. On clicking the numbers in the sample count, a pop up will open, detailing in brief about the samples and the details of the circRNAs in those samples.
- **Sample Score** : A metric used to measure the abundance of a circRNA. It tells in how many samples out of the total samples analysed the circRNA is found.
- **Evidence Type** : Represents what type of evidences were used to infer the circRNA-Protein Interactions(CPIs).
- **Tools**: The respective tools used to analyse the CPIs across all the samples overall.

# Explore circRNA Section

Just paste the circRNA ID and then click on the respective 4 tabs, as shown above, and then clicking on these respective tabs will give information for the respective section, for e.g., Get Proteins.

**circDiscoverer** A database for exploring human circRNA–protein interactions

Home Literature Section Predicted Section **Explore circRNA Section** Help Download

### Explore circRNA

Enter the circRNA ID:

**Get Proteins** **Get Guide RNAs** **Get Modifications** **Get Primers**

#### Proteins Associated with hsa-ARID1A\_0001

**Proteins with Motifs** **Proteins without Motifs**

Protein (RBP)	Samples	Motifs
IGF2BP3	<a href="#">View</a>	<a href="#">View</a>

**Figure 4**

# Explore circRNA Section – Get Proteins Details

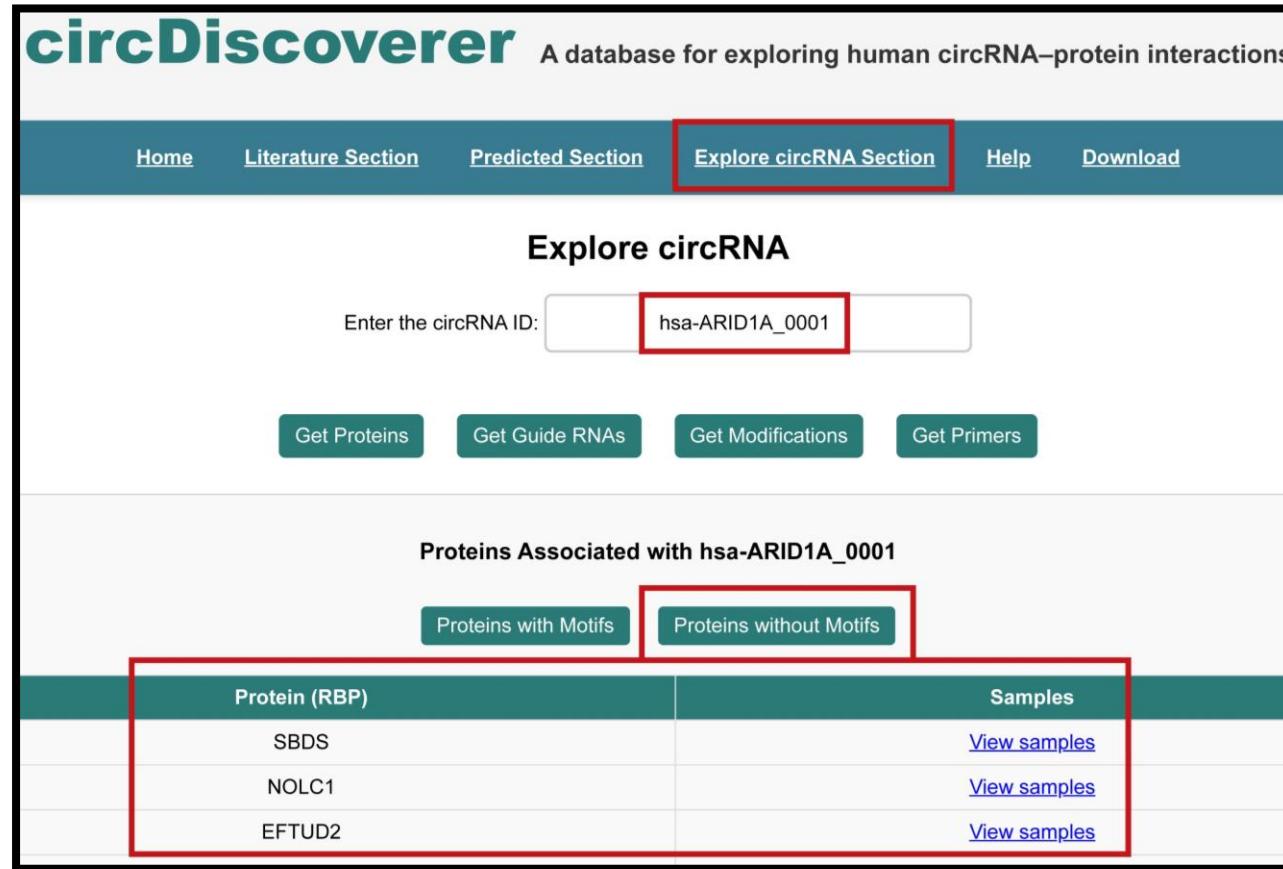
## Protein with Motifs

Clicking on the “**Protein with Motifs**” gives the list of proteins interacting with the circRNA of interest, having known RNA binding motifs.

**View** : Clicking on this opens a pop up, that displays the sample in which the circRNA-RBP interaction were found.

**Motifs** : Clicking on this opens a pop up, that displays details of the known RNA binding motifs of a protein in that circRNA.

# Protein without Motifs



**Explore circRNA**

Enter the circRNA ID:

Get Proteins Get Guide RNAs Get Modifications Get Primers

Proteins Associated with hsa-ARID1A\_0001

Proteins with Motifs **Proteins without Motifs**

Protein (RBP)	Samples
SBDS	<a href="#">View samples</a>
NOLC1	<a href="#">View samples</a>
EFTUD2	<a href="#">View samples</a>

**Figure 5**

Clicking on the “**Protein without Motifs**” gives the list of proteins interacting with the circRNA of interest, but with no RNA binding motifs known.

**View Samples** : Clicking on this opens a pop up, that displays the sample in which the circRNA-RBP interaction were found.

# Get Guide\_RNA

Gives list of Forward & the Reverse oligos for cloning the gRNA's in RfxCas13d expressing plasmid vector called pXR004

**circDiscoverer** A database for exploring human circRNA–protein interactions

Home Literature Section Predicted Section Explore circRNA Section Help Download

### Explore circRNA

Enter the circRNA ID: hsa-RP11-206L10\_0001

Get Proteins **Get Guide RNAs** Get Modifications Get Primers

Guide RNAs targeting hsa-RP11-206L10\_0001

NOTE: Check the off-target binding of these gRNA's.

Forward oligo(5'-3')	Reverse oligo(5'-3')	gRNA Spacer Length	RfxCas13d Plasmid Vector
AAACTTAAAGATATTGACCACATAAT	ATTATGTGGTCAAATATCTTAAAGTTC	23	<a href="#">pXR004</a>
AAACATTAAAGATATTGACCACATAA	TTATGTGGTCAAATATCTTAAATGTT	23	<a href="#">pXR004</a>
AAACTATTAAAGATATTGACCACATA	TATGTGGTCAAATATCTTAAATAGTTC	23	<a href="#">pXR004</a>

**Figure 6**

Clicking the guide RNA's will land into the NCBI-BLAST page with the sequence pasted directly, allowing users to check the off-targets in their own settings (Page 9), i.e.

# Guide\_RNA Results Page

Standard Nucleotide BLAST

blastn    blastp    blastx    tblastn    tblastx

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

**Enter Query Sequence**

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

>seq  
AAACTTTAAGATATTGACCACATAAT

Query subrange [?](#)

From

To

Or, upload file  Choose File No file chosen [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

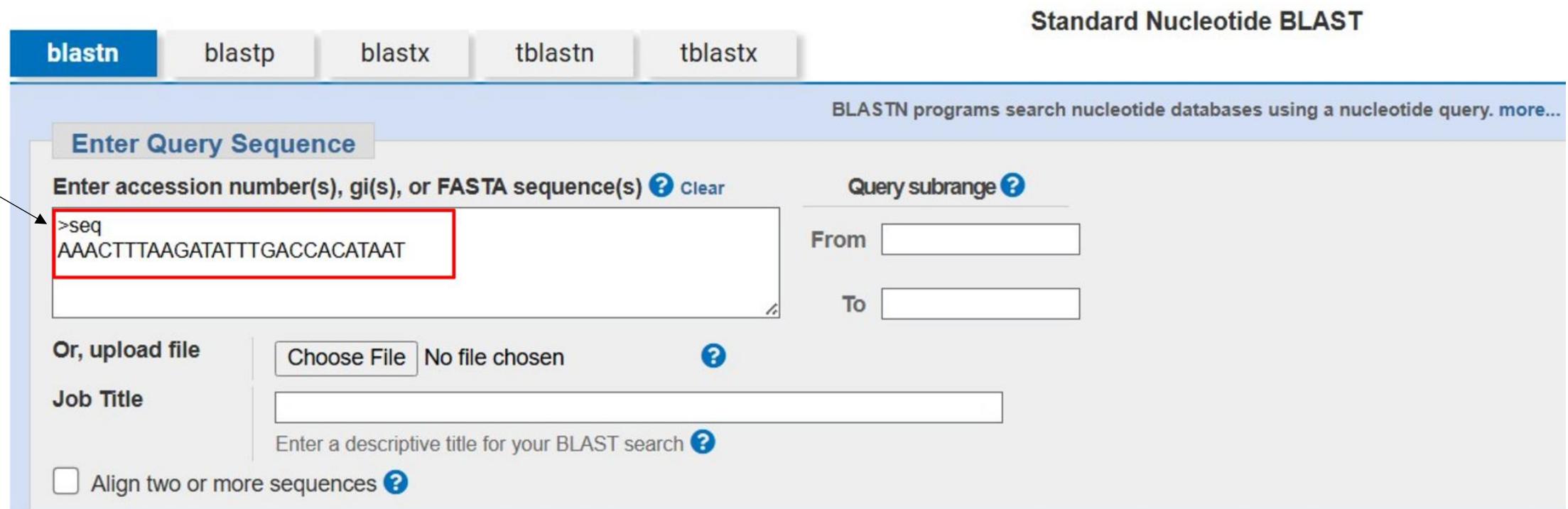
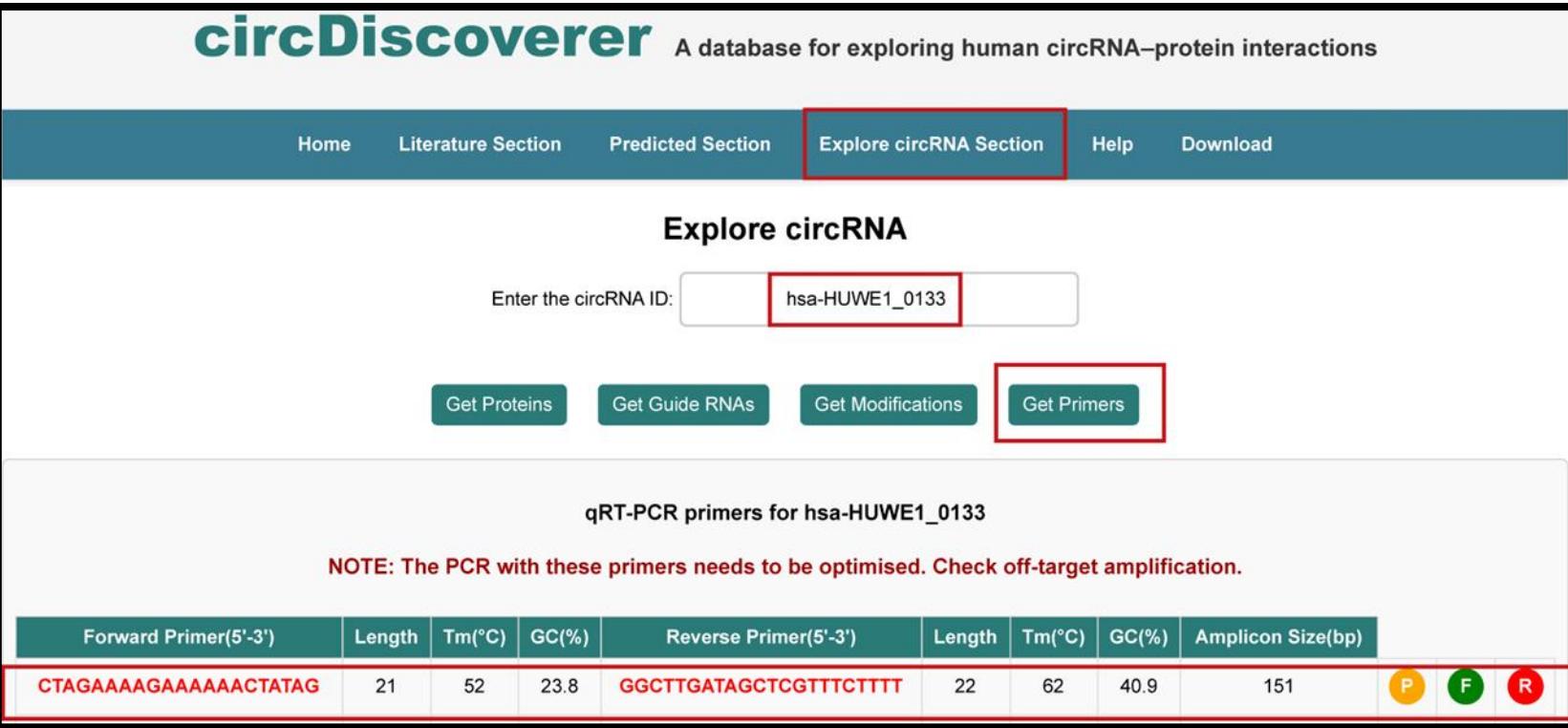


Figure 7

# Get Primers

Get primers amplifying the backsplicing junction



**circDiscoverer** A database for exploring human circRNA–protein interactions

Home Literature Section Predicted Section Explore circRNA Section Help Download

Explore circRNA

Enter the circRNA ID:

Get Proteins Get Guide RNAs Get Modifications **Get Primers**

qRT-PCR primers for hsa-HUWE1\_0133

NOTE: The PCR with these primers needs to be optimised. Check off-target amplification.

Forward Primer(5'-3')	Length	Tm(°C)	GC(%)	Reverse Primer(5'-3')	Length	Tm(°C)	GC(%)	Amplicon Size(bp)	P	F	R
CTAGAAAAGAAAAAACTATAG	21	52	23.8	GGCTTGATAGCTCGTTCTTT	22	62	40.9	151	P	F	R

Figure 8

Clicking the buttons, i.e. P(Primer pair), F(Forward primer) ,R(Reverse primer) lands onto the NCBI-primer BLAST page with the primer sequence pasted, where the users can check for the off-targets (Page 11-A,B,C).

# NCBI-Primer BLAST Page

A)

Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST).

Primers for target on one template

Primers common for a group of sequences

PCR Template

Enter accession, gi, or FASTA sequence (A refseq record is preferred)

Range

Forward primer

Reverse primer

Or, upload FASTA file  No file chosen

Primer Parameters

Use my own forward primer (5'-3' on plus strand)

Use my own reverse primer (5'-3' on minus strand)

CTAGAAAAGAAAAACTATAG

GGCTTGATAGCTCGTTCTTT

B)

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

Query subrange

From  To

>Reverse\_Primer  
GGCTTGATAGCTCGTTCTTT

Or, upload file  No file chosen

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

C)

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

Query subrange

From  To

>Forward\_Primer  
CTAGAAAAGAAAAACTATAG

Or, upload file  No file chosen

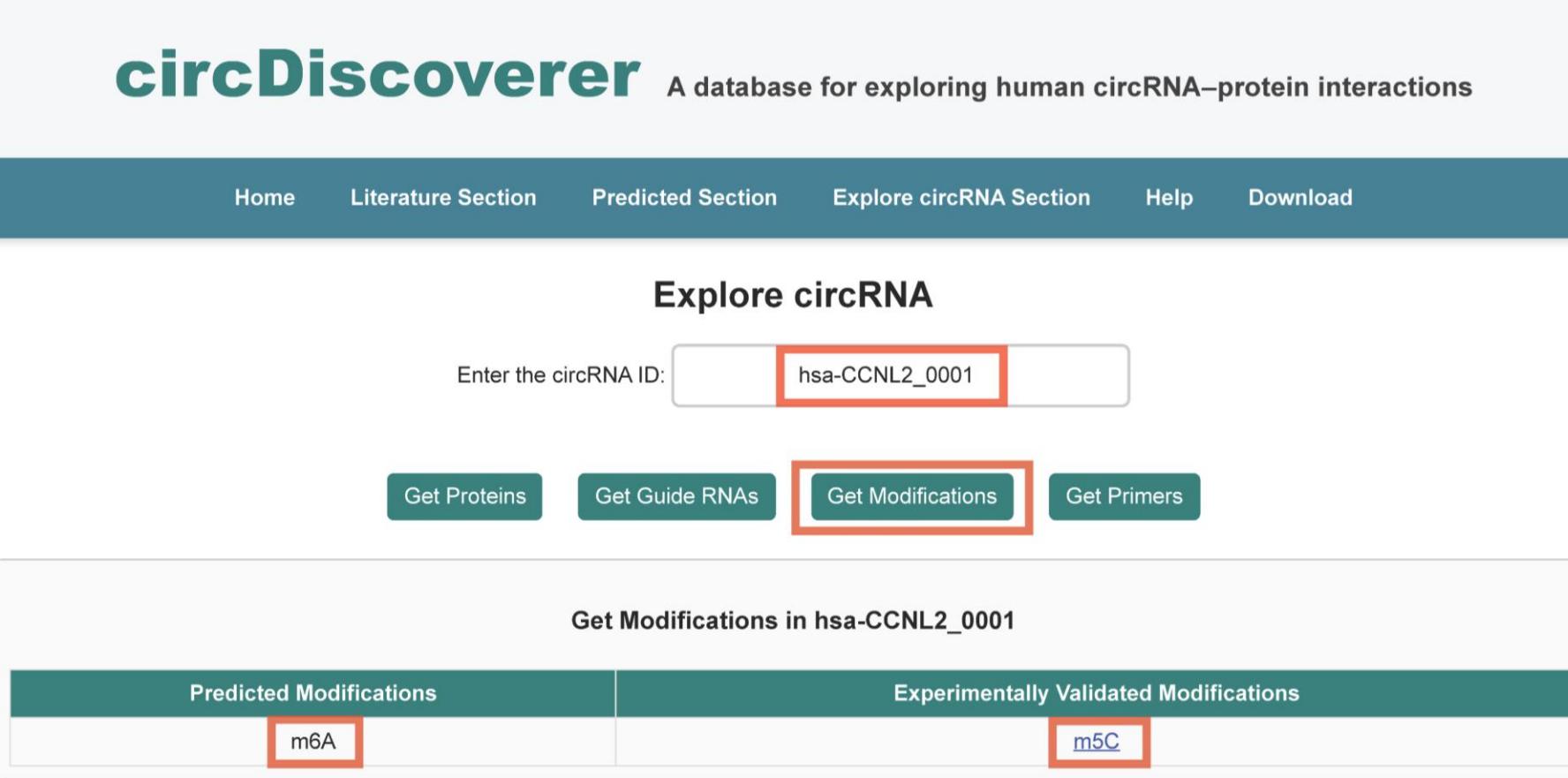
Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

# Get Modifications

Displays the possible modifications in a circRNA, alongwith the experimentally validated modifications.



**circDiscoverer** A database for exploring human circRNA–protein interactions

Home Literature Section Predicted Section Explore circRNA Section Help Download

Explore circRNA

Enter the circRNA ID:

Get Proteins Get Guide RNAs **Get Modifications** Get Primers

Get Modifications in hsa-CCNL2\_0001

Predicted Modifications	Experimentally Validated Modifications
m6A	<a href="#">m5C</a>

Clicking this hyperlinked Experimentally validated Modification will lead directly to the respective literature, and when clicked on Predicted Modification, a pop will open, detailing possible positions of the modification in circRNA, and the respective cell line where this modification can be found.

# qRT-PCR primers and gRNAs Designed with Non-Standard Rules

Some gRNAs and qRT-PCR Primers have been designed beyond the standard rules, such entries will be reflected with the respective warning as shown below, with the nucleotide sequences highlighted in red:

A)

**circDiscoverer** A database for exploring human circRNA–protein interactions

Home Literature Section Predicted Section **Explore circRNA Section** Help Download

**Explore circRNA**

Enter the circRNA ID:

Get Proteins **Get Guide RNAs** Get Modifications Get Primers

**Guide RNAs targeting hsa-ADAMTSL1\_0139**

NOTE: The gRNA's contain mismatches! Check the off-target binding of these gRNA's.

Forward oligo(5'-3')	Reverse oligo(5'-3')	gRNA Spacer Length	RfxCas13d Plasmid Vector
AAACCTCCGCATACAATTGCAGTAAG	CTTACTGCAAATTGTATGCGGAGGTTTC	23	pXR004
AAACTCTCCGCATACAATTAGCGTAA	TTACGCTAAATTGTATGCGGAGAGTTC	23	pXR004

B)

**circDiscoverer** A database for exploring human circRNA–protein interactions

Home Literature Section Predicted Section **Explore circRNA Section** Help Download

**Explore circRNA**

Enter the circRNA ID:

Get Proteins **Get Guide RNAs** Get Modifications **Get Primers**

**qRT-PCR primers for hsa-RP11-206L10\_0001**

NOTE: The PCR with these primers needs to be optimised. Check the off-target amplification of these primer pairs.

Forward Primer(5'-3')	Length	Tm(°C)	GC(%)	Reverse Primer(5'-3')	Length	Tm(°C)	GC(%)	Amplicon Size(bp)
AAAGAAAGGCATGGCTATTGCA	22	62	40.9	TTTGTGTCAATTCTAAATGGT	22	56	27.3	195
GCTATTGCACCTGGAGAA	20	60	50	AAAATGGTCCTTTTCCTT	20	52	30	169

# HELP Page

## circDiscoverer

A database for exploring human circRNA–protein interactions

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circDiscoverer Tutorial  
[!\[\]\(fc5cbba58387ad7b76029814c568029c\_img.jpg\) Click here to open the Tutorial \(PDF\)](#)

Contact the following persons

**IISER-Bhopal**  
Dr. Ajit Chande  
[ajitg@iiserb.ac.in](mailto:ajitg@iiserb.ac.in)

**THSTI-Faridabad**  
Prof. Samrat Chatterjee  
[samrat.chatterjee@thsti.res.in](mailto:samrat.chatterjee@thsti.res.in)

**Sreeram Srinivasan**  
[sreeramkv2@gmail.com](mailto:sreeramkv2@gmail.com)

**Dr. Shivam Kumar**  
[shvmkmr15@gmail.com](mailto:shvmkmr15@gmail.com)