SUPPLEMENTAL TEXT

CircInteractome: a web tool for exploring circular RNAs and their interacting proteins and microRNAs

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Running title: CircInteractome: circRNA-binding partners

Key words: RNA-binding proteins; sponge circRNAs; divergent primer design; circRNA IRES; transcriptome; circRNA-miRNA; CLIP-Seq; circRNA siRNA

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Supplementary Figures and Tables

Supplementary Figure S1	RBPs binding to circRNAs
Supplementary Figure S2	Genomic and mature sequence of circRNAs
Supplementary Figure S3	CircRNA binding sites for a given interacting RBP
Supplementary Figure S4	RBP-binding site on circRNA junction and flanking sequence
Supplementary Figure S5	miRNA-circRNA interactions predicted by CircInteractome
Supplementary Figure S6	Divergent primer design output
Supplementary Figure S7	Design of siRNAs directed at circRNA junction
Supplementary Table S1	RBP datasets used in this study
Supplementary Table S2	RBPs interacting with circRNAs
Supplementary Table S3	IRES in circRNAs
Supplementary Table S4	miRNA super-sponge circRNAs

Figure S1. RBPs binding to circRNAs. A. RBPs binding to mature circRNAs. **B.** Relative number of circRNAs targeted by any RBP on the junction compared to the number of circRNA targeted at mature circRNA sequence. **C.** Ratio of number of circRNAs targeted by RBPs on flanking sequences of circRNA relative to mature circRNA.

Figure S2. Genomic and mature sequences of a given circRNA. Illustrative screenshots from the CircInteractome showing the genomic (*top*) and mature circRNA (*bottom*) sequence for hsa_ circ_0000020. Blue line indicates a break in the sequence.

Figure S3: CircRNA binding sites for a given interacting RBP. A. RBP (HNRNPC) binding sites on all reported circRNAs. **B.** Search for specific RBP (HNRNPC) binding site on any circRNA (hsa_circ_00000143).

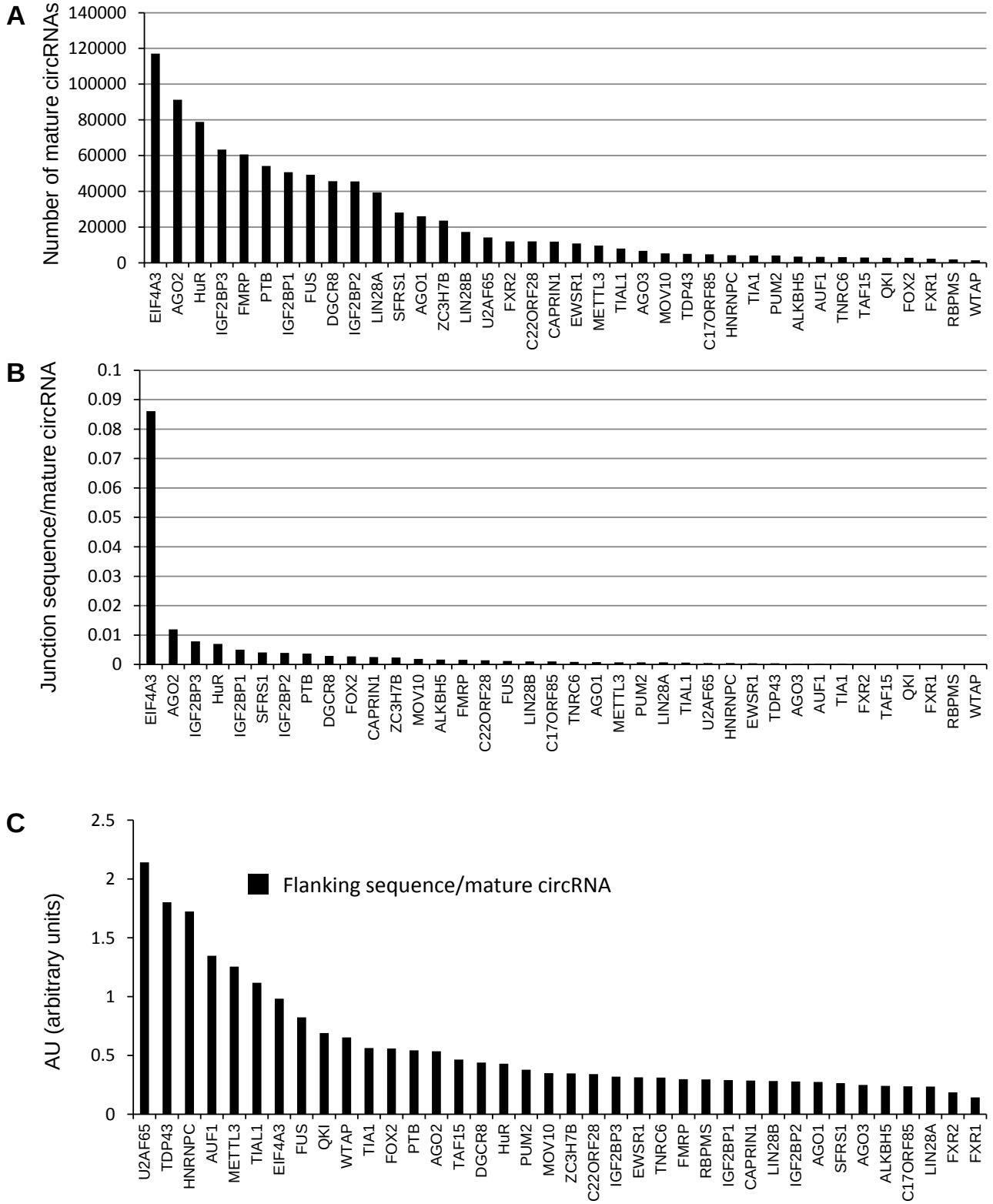
Figure S4. RBP-binding site on circRNA junction and flanking sequence. A. Details of EIF4A3 binding sites on hsa_circ_0000020 junction sequence. **B.** Details of EIF4A3 binding sites on hsa_circ_0000020 flanking sequence.

Figure S5. miRNA-circRNA interactions predicted by CircInteractome.

Figure S6. Divergent primer design output. A. Divergent primer design output file for has_circ_0000020 using NCBI primer design tool. **B.** Divergent primer design output file for has_circ_0000020 using Primer3 (v. 0.4.0) primer design tool.

Figure S7. Design of siRNAs directed at circRNA junction. The numbers in the parentheses represent the priority. To finalize the sequence, the user needs to add dinucleotide (dTdT) 3'DNA overhangs to increase the efficiency of the siRNA.

Dudekula, Panda et al., Suppl. Fig. S1



Dudekula, Panda et al., Suppl. Fig. S2

Home
Circular RNA
RBP on CircRNA
miRNA Target Sites
Divergent Primers
siRNA Design
Help

hsa_circ_0000020 Circular RNA Genomic Sequence

GCAACAGAACATTGAGGAAAACATGACAATAGCTATGGAAGAGGCTCCGGAAAGTTTTGGCCAAGTAGTGATGCTTTATA
TTAACTGCAAAGTGAATGGACATCCTGTGAAAGCCTTTGTTGACTCAGGTGACGTCTCTGTCTTTTATTTCTTGGTCTCC
CCTCCAACATCTTTACCTGACACGGGAGAAGGGGAGGCTCAAGCATCCGTTCAGATGGCTGTACTTTTTTTTTTTTTTTT
TTTTTTGAGACAGAGTCTTGCTCTGTCACCCAGGCCAAAGTGCAGTGGCGTGATCTTGGCTCACTGCAACCTCTGTCTCC
TCAGTTCAAGCGATTCTCCAGCCTCGGCCTCCCACGTAGCTGGGATTACAGGCGCCAACCACCACGCCCAGCTGATTTTT
ACATTTTTAGTAGAGATGGGGTTTCACCGTGTTGGTCAGGCTGGTCTTGAACTCCTGACCTCAAGTGATCCACCGTGCCT
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TATTAGGTTTCTTTCTTTAATCATTTCTTAACCAGTTTTGTATTTTGTGGTGTCTCCTTTTTCTTCCTAACTCCCCTTAT
CTAGCATCCTGAATTTCTTGGATGTTTCTGTAAAAAACTGGGAATACATAGGTTTAGAAGAAGGAGCTATAAAAGGAGCT
GTGTAGAGTATAGGTCAAACACATTTCAGCCTGGCCTGTGCACCAGCACTTTGCCAAGATTTGGGAGGAGTGTAAGGCTA
TCTGGGTATGGTTCTTACCCTTAAAGAACTTAACAACCAAGTAAGGAGACAGGGATTCTTCTGCTTGAGGGTTCAGTTAA
TTCACATACTAGTTGGCACCTTTTCTGTCAGTAACAACCGTTTGCTTTTTAGTAAGACTACACCATGCAGCATTTTGTGG
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GAAAAATTCCTTGCTCAAATAATATTTTCCCCCTTCACTGTGTATTTCTATATAAAAAGAAAAAAAGTATTTTCTTTCAT
TTCTTCATCTACACATTGCTCACGACATCGTCCCTTTCACGTCTCTCTCTCTCATTTTTTAAACACACCCTCTCTCTCAC
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CCTCCCAAAGTGCCGGGATTACAGGCGTGAGCCACCACGCCCGGCTCACAGCATTCTAAATAGAACGAATGAATGAATGA
GTGTATGTATCAATAAAACCTTGTCACTGCCTTTCCTTATTTTTTTAGTCATGGTATAGACCAGCAGTCTCCAACTTTAT
AGATGTAAATCATATATGTGCACAACTGTCTATTAAGTAGTTATAAAATACACAAAAAAGGAAGTTAGCATTAGCATGGA
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TCCTGCCTCAGCCTCCCAAGTAGCTGCGATTATGGGCATGTGCCACCACGCCTGGTTAATTTTTGTATTTTTAGTAGAGG
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GGGATTACAGGCATGAGTCACCGTGCCTGGCCATTTGTGATTGGAGTTTTAAATTAAGGAGCATAATTTTTCTGCATGGT
GTTTTATCTGCTATTATTCTTTGGTTTCTTTCTTCCAAGTGTTCCATCGACCTGAAGAAAAATGTACTCGTGATCGGCAC
CACAGGCTCCCAGACCACCTTTCTTCCTGAGGGAGAGCTACCAGAGTGTGCCCGGTTGGCATATGGGGCTGGAAGAGAGG
ATGTACGGCCAGAGGAGATTGCAGACCAAGAATTAGCAGAAGCCCTTCAAAAATCAGCAGAGGATGCAG

Length: 13589 bp

Home
Circular RNA
RBP on CircRNA
miRNA Target Sites
Divergent Primers
siRNA Design
Help

hsa_circ_0000020 Circular RNA Mature Sequence

GCAACAGAACATTGAGGAAAACATGACAATAGCTATGGAAGAGGCTCCGGAAAGTTTTGGCCAAGTAGTGATGCTTTATA
TTAACTGCAAAGTGAATGGACATCCTGTGAAAGCCTTTGTTGACTCAGGTGCCCAGATGACTATCATGAGCCAAGCTTGT
GCAGAAAGGTGTAACATAATGAGACTGGTGGACCGTCGGTGGGCAGGGATTGCCAAAGGAGTGGGCACCCAGAAGATTAT
TGGAAGGGTACATCTAGCTCAGGTTCAGATTGAAGGAGATTTTTTGCCATGTTCCTTCTCTATACTTGAGGAACAGCCCA
TGGACATGCTTCTGGGACTGGACATGCTTAAACGGCACCAGTGTTCCATCGACCTGAAGAAAAATGTACTCGTGATCGGC
ACCACAGGCTCCCAGACCACCTTTCTTCCTGAGGGAGAGCTACCAGAGTGTGCCCGGTTGGCATATGGGGCTGGAAGAGA
GGATGTACGGCCAGAGGAGATTGCAGACCAAGAATTAGCAGAAGCCCTTCAAAAATCAGCAGAGGATGCAG

Length: 551 bp

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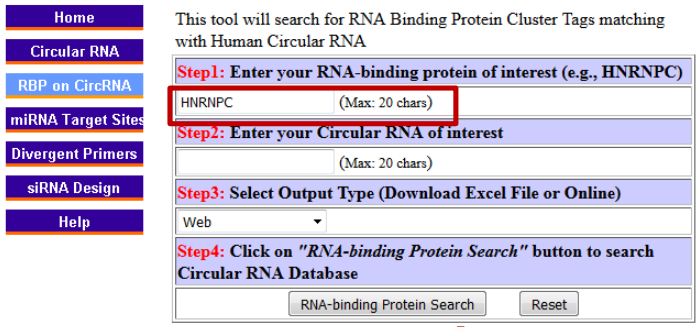
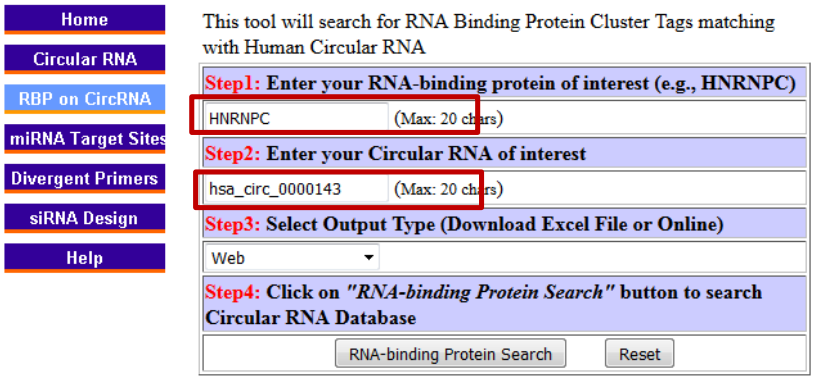
Dudekula, Panda et al., Suppl. Fig. S3

A



Circular RNA	Tag Name	% Identity	Alignment Length	Mismatches	Gap Openings	Tag Start	Tag End	Circular RNA Start	Circular RNA ENd
hsa_circ_0000003	HIUHC_262311_HNRNPC_262311_33	93.94	33	2	0	1	33	19958	19990
hsa_circ_0000003	HIUHC_210_HNRNPC_210_34	100.00	34	0	0	1	34	6622	6655
hsa_circ_0000004	HIUHC_215_HNRNPC_215_24	100.00	24	0	0	1	24	10694	10717
hsa_circ_0000004	HIUHC_235_HNRNPC_235_23	100.00	23	0	0	1	23	26215	26237
hsa_circ_0000004	HIUHC_210_HNRNPC_210_34	100.00	34	0	0	1	34	6222	6255
hsa_circ_0000005	HIUHC_168762_HNRNPC_168762_30	100.00	29	0	0	2	30	52193	52221
hsa_circ_0000005	HIUHC_338_HNRNPC_338_22	100.00	22	0	0	1	22	61536	61557
hsa_circ_0000006	HIUHC_344_HNRNPC_344_29	100.00	29	0	0	1	29	3234	3262
hsa_circ_0000068	HIUHC_10142_HNRNPC_10142_72	100.00	72	0	0	1	72	531	602
hsa_circ_0000090	HIUHC_175899_HNRNPC_175899_32	93.75	32	2	0	1	32	7329	7360
hsa_circ_0000143	HIUHC_398200_HNRNPC_398200_35	97.14	35	1	0	1	35	22414	22448
hsa_circ_0000143	HIUHC_389596_HNRNPC_389596_26	100.00	26	0	0	1	26	33031	33056
hsa_circ_0000143	HIUHC_325371_HNRNPC_325371_38	94.59	37	2	0	1	37	34013	34049
hsa_circ_0000169	HIUHC_436360_HNRNPC_436360_29	100.00	28	0	0	1	28	4586	4613
hsa_circ_0000169	HIUHC_346851_HNRNPC_346851_28	100.00	28	0	0	1	28	4742	4769

B



Circular RNA	Tag Name	% Identity	Alignment Length	Mismatches	Gap Openings	Tag Start	Tag End	Circular RNA Start	Circular RNA ENd
hsa_circ_0000143	HIUHC_325371_HNRNPC_325371_38	94.59	37	2	0	1	37	34013	34049
hsa_circ_0000143	HIUHC_389596_HNRNPC_389596_26	100.00	26	0	0	1	26	33031	33056
hsa_circ_0000143	HIUHC_398200_HNRNPC_398200_35	97.14	35	1	0	1	35	22414	22448

Dudekula, Panda et al., Suppl. Fig. S4

A

RNA-binding protein sites matching Circular RNA junction	
RNA-binding Protein	**# Tags**
EIF4A3	1

Circular RNA	Tag Name	% Identity	Alignment Length	Mismatches	Gap Openings	Tag Start	Tag End	Circular RNA Start	Circular RNA ENd
hsa_circ_0000020 Length: 551 bp	HHLE1_1987_eIF4AIII_rep1_1987_16_76	100.00	76	0	0	1	76	-3	+73

B

RNA-binding protein sites matching flanking regions of Circular RNA	
RNA-binding Protein	**# Tags**
EIF4A3	4

Circular RNA	Tag Name	% Identity	Alignment Length	Mismatches	Gap Openings	Tag Start	Tag End	Circular RNA Start	Circular RNA ENd	Upstream/Downstream
hsa_circ_0000020	HHLE1_1987_eIF4AIII_rep1_1987_16_76	100.00	76	0	0	1	76	-3	73	Upstream
hsa_circ_0000020	HHLE2_6580_eIF4AIII_rep2_6580_1_34	100.00	34	0	0	1	34	-518	-485	Upstream
hsa_circ_0000020	HHLE2_6581_eIF4AIII_rep2_6581_2_30	100.00	30	0	0	1	30	-263	-234	Upstream
hsa_circ_0000020	HHLE2_6582_eIF4AIII_rep2_6582_35_138	100.00	138	0	0	1	138	-7	131	Upstream

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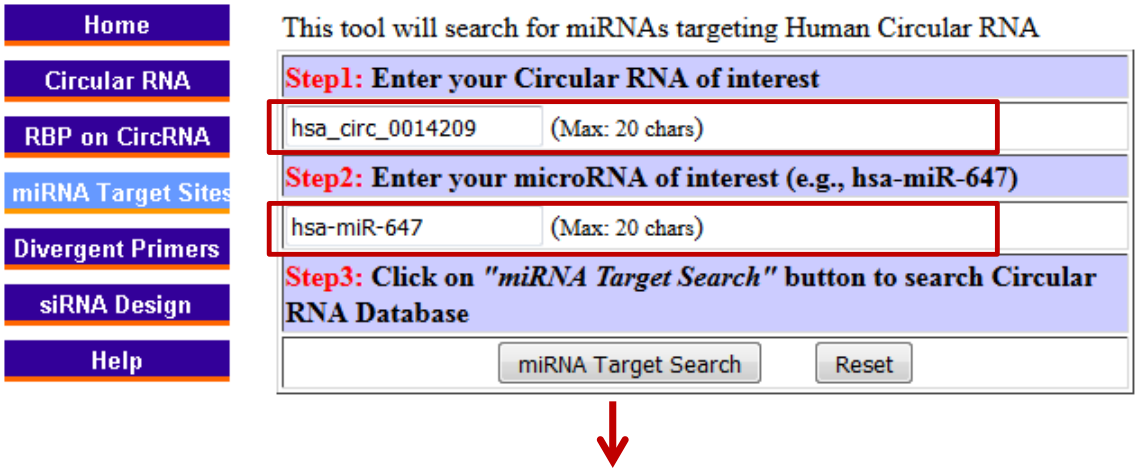


TargetScan miRNA predictions											
CircRNA Mirbase ID	CircRNA (Top) - miRNA (Bottom) pairing	Site Type	CircRNA Start	CircRNA End	3' pairing	local AU	position	TA	SPS	context+ score	context+ score percentile
hsa_circ_0014209 (5' ... 3') hsa-miR-647 (3' ... 5')	AGAGAAGCAGCAGGGGCAGCCAA CUUCCUUCACUCA---CGUCGGUG	8mer-1a	1189	1196	-0.018	0.116	0.123	0.018	-0.144	-0.152	77
hsa_circ_0014209 (5' ... 3') hsa-miR-647 (3' ... 5')	AGAGCAGCAGGUGGGGCAGCCAA CUUCCUUCACUCACGUCGGUG	8mer-1a	1420	1427	-0.008	0.106	0.065	0.018	-0.144	-0.21	89
hsa_circ_0014209 (5' ... 3') hsa-miR-647 (3' ... 5')	CAGGAGCAAAUGAAACAGCCAAC CUUCCUUCACUCACGUCGGUG	7mer-1a	152	158	-0.002	0.022	-0.033	0.010	-0.063	-0.14	74
hsa_circ_0014209 (5' ... 3') hsa-miR-647 (3' ... 5')	GAGCAGCAGGUAGGACAGCCAAA CUUCCUUCACUCACGUCGGUG	7mer-1a	1541	1547	0.001	0.043	0.014	0.010	-0.063	-0.069	53
hsa_circ_0014209 (5' ... 3') hsa-miR-647 (3' ... 5')	GGAUCAGCAGGAGAAGCAGCCAG CUUCCUUCACUCACGUCGGUG	7mer-m8	949	955	0.003	0.026	0.073	0.010	-0.084	-0.092	60

Dudekula, Panda et al., Suppl. Fig. S6A

Primer3 Output

PRIMER PICKING RESULTS FOR hsa_circ_0000020

No mispriming library specified
Using 1-based sequence positions

OLIGO	start	len	tm	gc%	any	3'	seq
LEFT PRIMER	26	20	59.83	60.00	4.00	0.00	GAGAGGATGTACGGCCAGAG
RIGHT PRIMER	157	20	59.83	50.00	6.00	1.00	AAACTTTCCGGAGCCTCTTC

SEQUENCE SIZE: 200
INCLUDED REGION SIZE: 200

PRODUCT SIZE: 132, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00

```
  1 GCCCGGTTGGCATATGGGGCTGGAAGAGAGGATGTACGGCCAGAGGAGATTGCAGACCAA
                >>>>>>>>>>>>>>>>>>>>

 61 GAATTAGCAGAAGCCCTTCAAAAATCAGCAGAGGATGCAGGCAACAGAACATTGAGGAAA


121 ACATGACAATAGCTATGGAAGAGGCTCCGGAAAGTTTTGGCCAAGTAGTGATGCTTTATA
            <<<<<<<<<<<<<<<<<<<

181 TTAACTGCAAAGTGAATGGA
```

KEYS (in order of precedence):
>>>>>> left primer
<<<<<< right primer

ADDITIONAL OLIGOS

	start	len	tm	gc%	any	3'	seq
1 LEFT PRIMER	25	20	59.83	55.00	4.00	2.00	AGAGAGGATGTACGGCCAGA
RIGHT PRIMER	157	20	59.83	50.00	6.00	1.00	AAACTTTCCGGAGCCTCTTC
PRODUCT SIZE: 133, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 3.00							
2 LEFT PRIMER	25	20	59.83	55.00	4.00	2.00	AGAGAGGATGTACGGCCAGA
RIGHT PRIMER	159	20	60.73	50.00	6.00	3.00	CAAAACTTTCCGGAGCCTCT
PRODUCT SIZE: 135, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 3.00							
3 LEFT PRIMER	17	20	59.24	55.00	2.00	2.00	GGGCTGGAAGAGAGGATGTA
RIGHT PRIMER	157	20	59.83	50.00	6.00	1.00	AAACTTTCCGGAGCCTCTTC
PRODUCT SIZE: 141, PAIR ANY COMPL: 6.00, PAIR 3' COMPL: 2.00							
4 LEFT PRIMER	31	20	59.12	55.00	4.00	2.00	GATGTACGGCCAGAGGAGAT
RIGHT PRIMER	157	20	59.83	50.00	6.00	1.00	AAACTTTCCGGAGCCTCTTC
PRODUCT SIZE: 127, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 2.00							

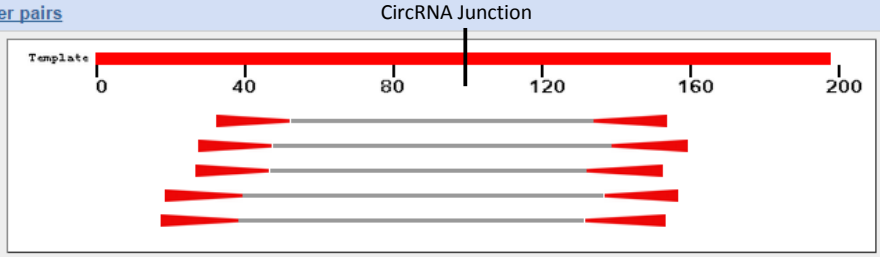
Dudekula, Panda et al., Suppl. Fig. S6B

Primer-BLAST — Primer-Blast results

NCBI/ Primer-BLAST : results: Job id=FQ7kV65dB3k4SzxFXWsOO0ZGPCpVWSEv more...

Input PCR template	hsa_circ_0000020
Range	1 - 200
Specificity of primers	Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports	Search Summary

Summary of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GTACGGCCAGAGGAGATTGC	Plus	20	34	53	60.53	60.00	4.00	2.00
Reverse primer	CTTTCCGGAGCCTCTTCCAT	Minus	20	154	135	59.46	55.00	6.00	2.00
Product length	121								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AGGATGTACGGCCAGAGGAG	Plus	20	29	48	60.76	60.00	4.00	0.00
Reverse primer	CCAAAACTTTCCGGAGCCTCT	Minus	21	160	140	60.55	52.38	6.00	3.00
Product length	132								

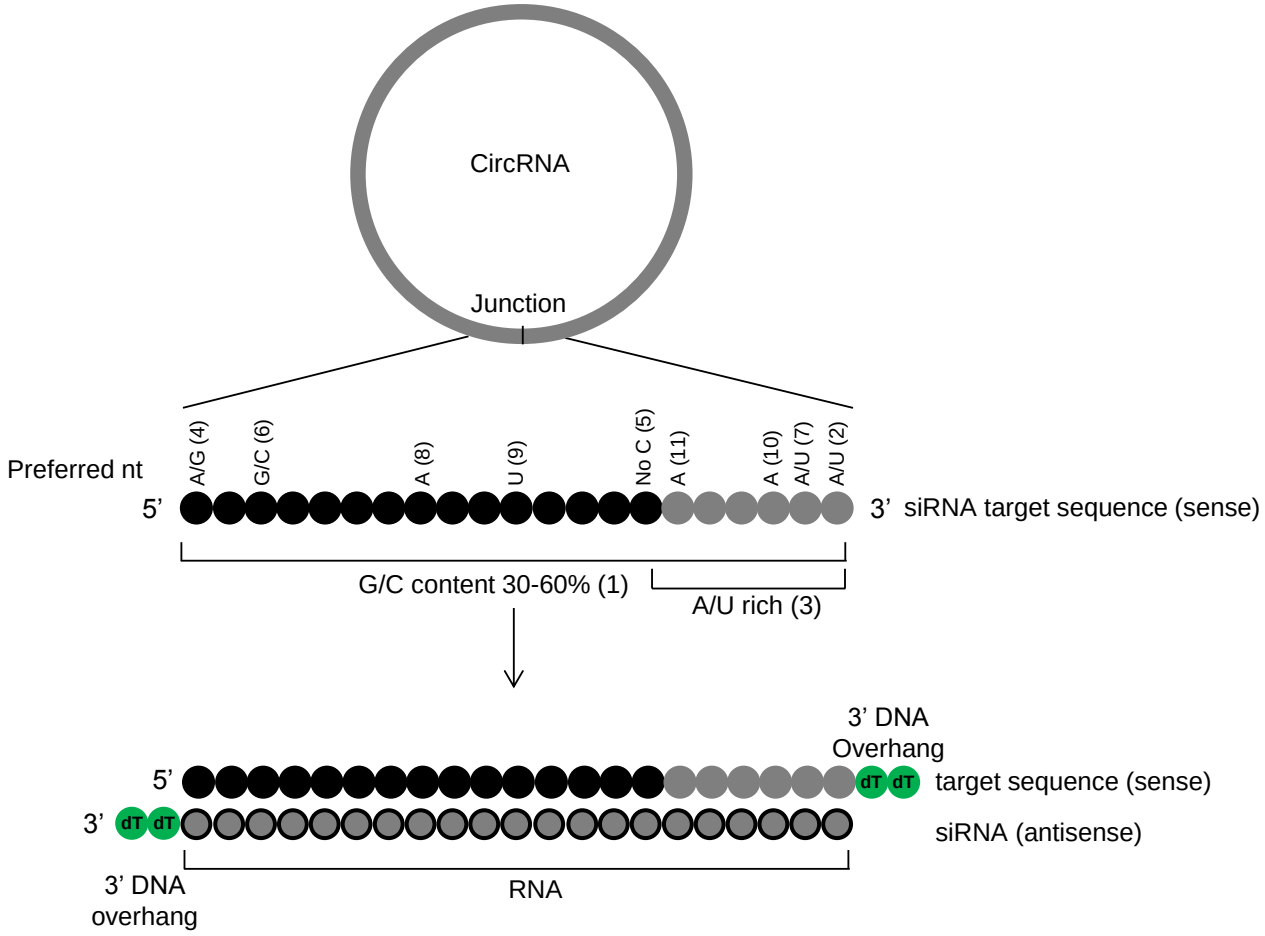
Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GAGGATGTACGGCCAGAGGA	Plus	20	28	47	60.76	60.00	4.00	0.00
Reverse primer	TTTCCGGAGCCTCTTCCATAG	Minus	21	153	133	59.24	52.38	6.00	2.00
Product length	126								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTGGAAGAGAGGATGTACGGC	Plus	21	20	40	59.93	57.14	4.00	2.00
Reverse primer	AAACTTTCCGGAGCCTCTTC	Minus	20	157	138	57.81	50.00	6.00	1.00
Product length	138								

Primer pair 5

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCTGGAAGAGAGGATGTACGG	Plus	21	19	39	59.93	57.14	4.00	1.00
Reverse primer	CTTTCCGGAGCCTCTTCCATAG	Minus	22	154	133	60.22	54.55	6.00	2.00
Product length	136								

Dudekula, Panda et al., Suppl. Fig. S7