Supporting Information

In vitro labeling strategies for *in cellulo* fluorescence microscopy of ribonucleoprotein machines

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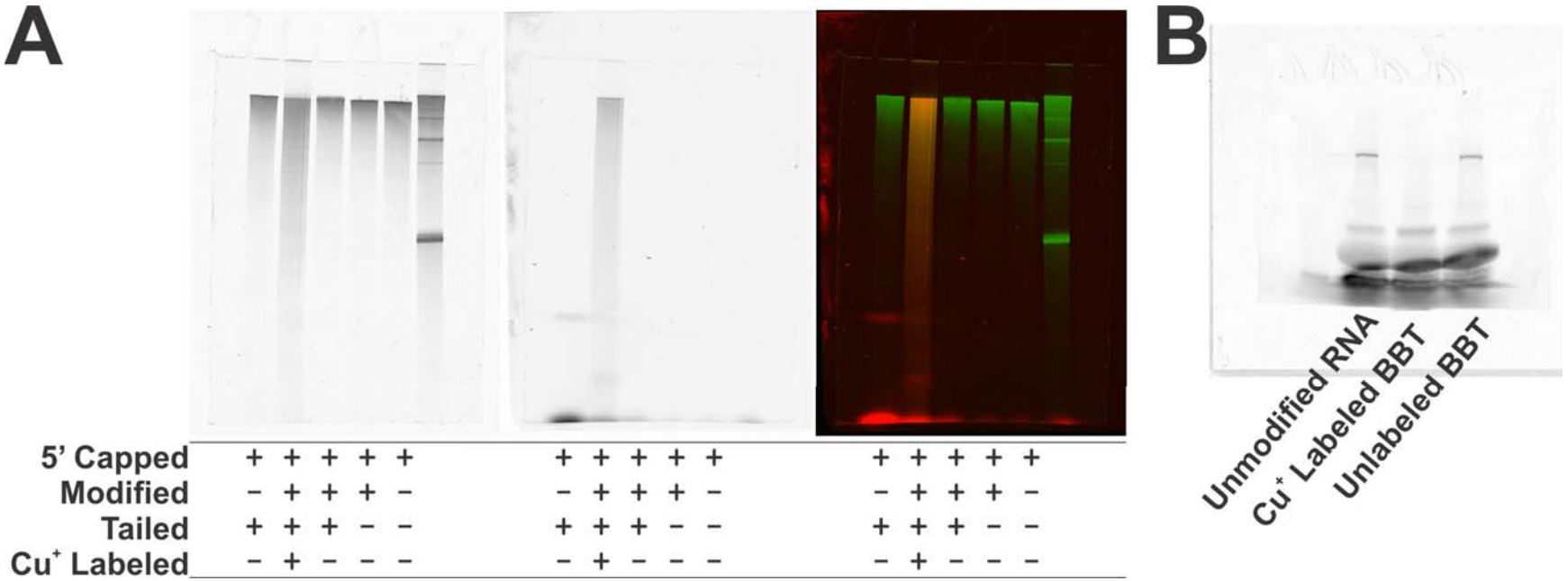

Figure S1. Copper based click chemistry approach degrades BBT modified mRNA. (A) 4% denaturing PAGE analysis of each step of the BBT labeling process using copper based click chemistry. The gel is scanned for the SYBR Gold staining of the FLuc mRNA (left panel, green) and for the presence of Alexa647 labeling (middle panel, red). The overlay of the two scans (right pane) indicates that the labeling process results in excessive degradation, or smearing of the RNA band. (B) SDS-PAGE analysis of a rabbit reticulocyte *in vitro* translation assay of unlabeled FLuc mRNA (lane 1), unlabeled BBT modified (lane 3) and copper click labeled BBT modified mRNA (lane 2).

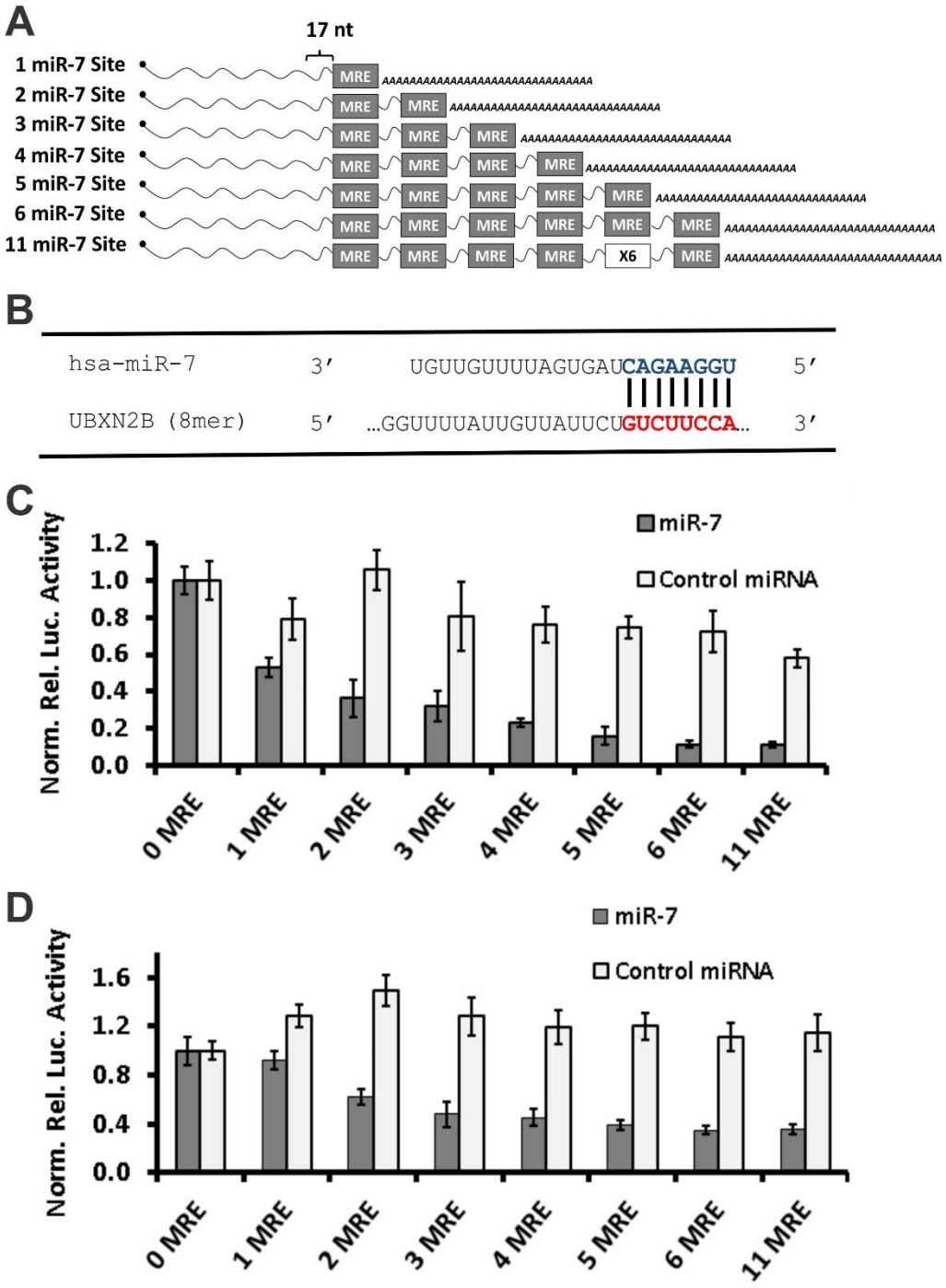

Figure S2. microRNA regulation element (MRE) dependent repression of FLuc expression. (A) Schematic representation of our cloned FLuc reporter genes containing 1 to 6 or 11 miR-7 MRE sites. Each MRE is separated by a 17 nucleotide (nt) spacer to accommodate multiple Ago binding events on a single transcript.[1] (B) Sequence alignment of miR-7 seed sequence (blue) and the complementary UBXN2B MRE target site (red) as predicted by TargetScan.[2] Relative luciferase response of transfected dual luciferase reporter plasmids in HeLa (C) and DCP1a-GFP stably expressing U2OS cells (D). FLuc reporter genes contained either 0-6 or 11 miR-7 MRE sites. Plasmids were co-transfected with either 100 nM duplexed miR-7 or control miRNA.

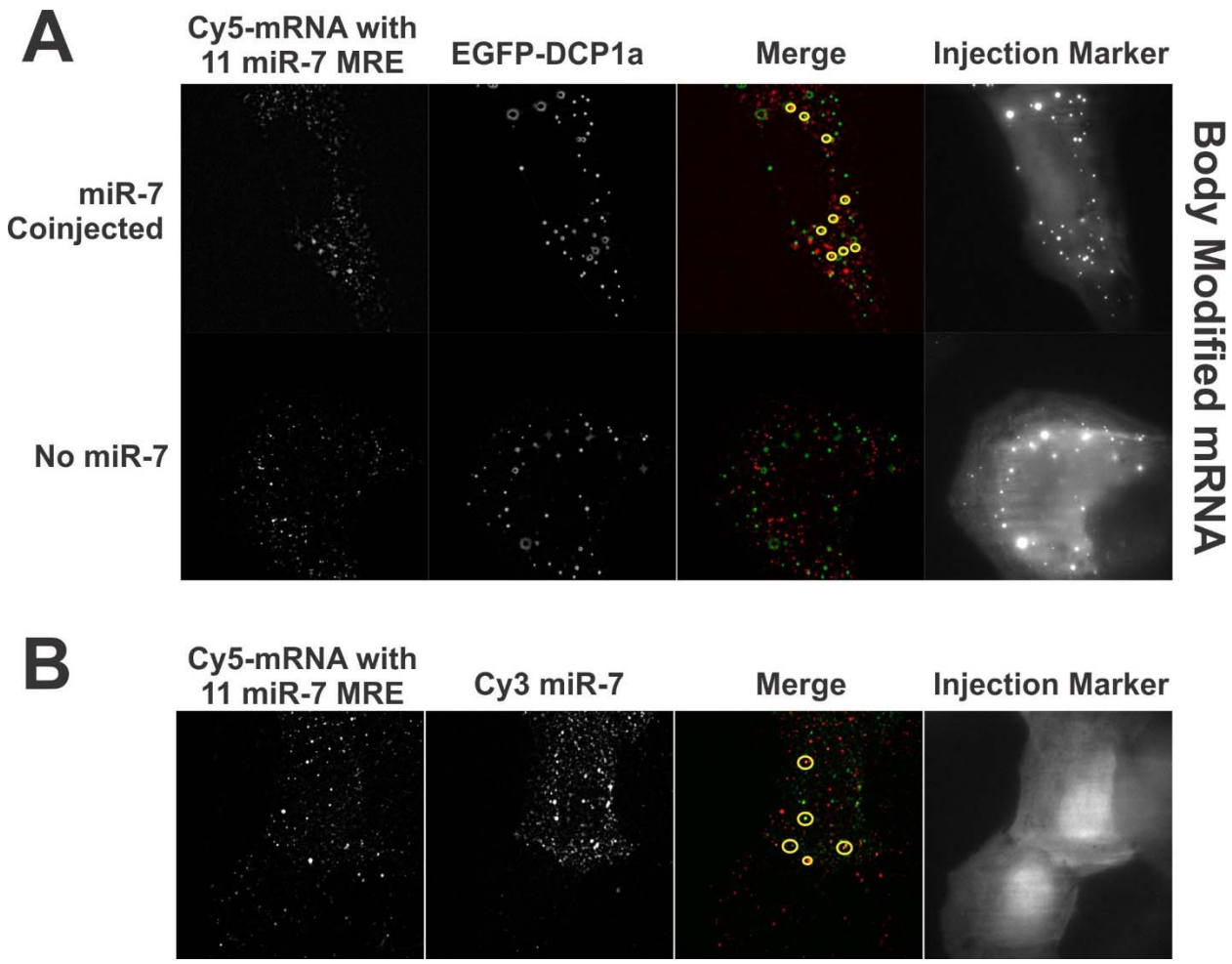

Figure S3. Cy5-body labeled mRNAs colocalize with P-Body marker DCP1a-EGFP and Cy3-labeled miR-7. (A) Fixed cell analysis of microinjected Cy5-body labeled FLuc mRNA, containing 11 miR-7 MRE sites, 2 h after microinjection into DCP1a-EGFP stably transfected U2OS cells. Yellow circles indicate sites of colocalization between DCP1a-EGFP (green) and RNA (red) coinjected with (top panels) or without miR-7 duplex (bottom panels). Cascade Blue-labeled 10 kDa Dextran was used as cell injection marker. (B) Fixed cell analysis of Microinjected Cy5-body labeled FLuc mRNA, containing 11 miR-7 MRE sites, and 3' labeled Cy3-labeled miR-7 guide strand duplexed with 5' Iowa Black® RQ labeled passenger strand. Colocalization between the mRNA (red) and miRNA (green) is indicated by yellow circles. Cascade Blue-labeled 10 kDa Dextran was used as a cell injection marker to identify treated cells.

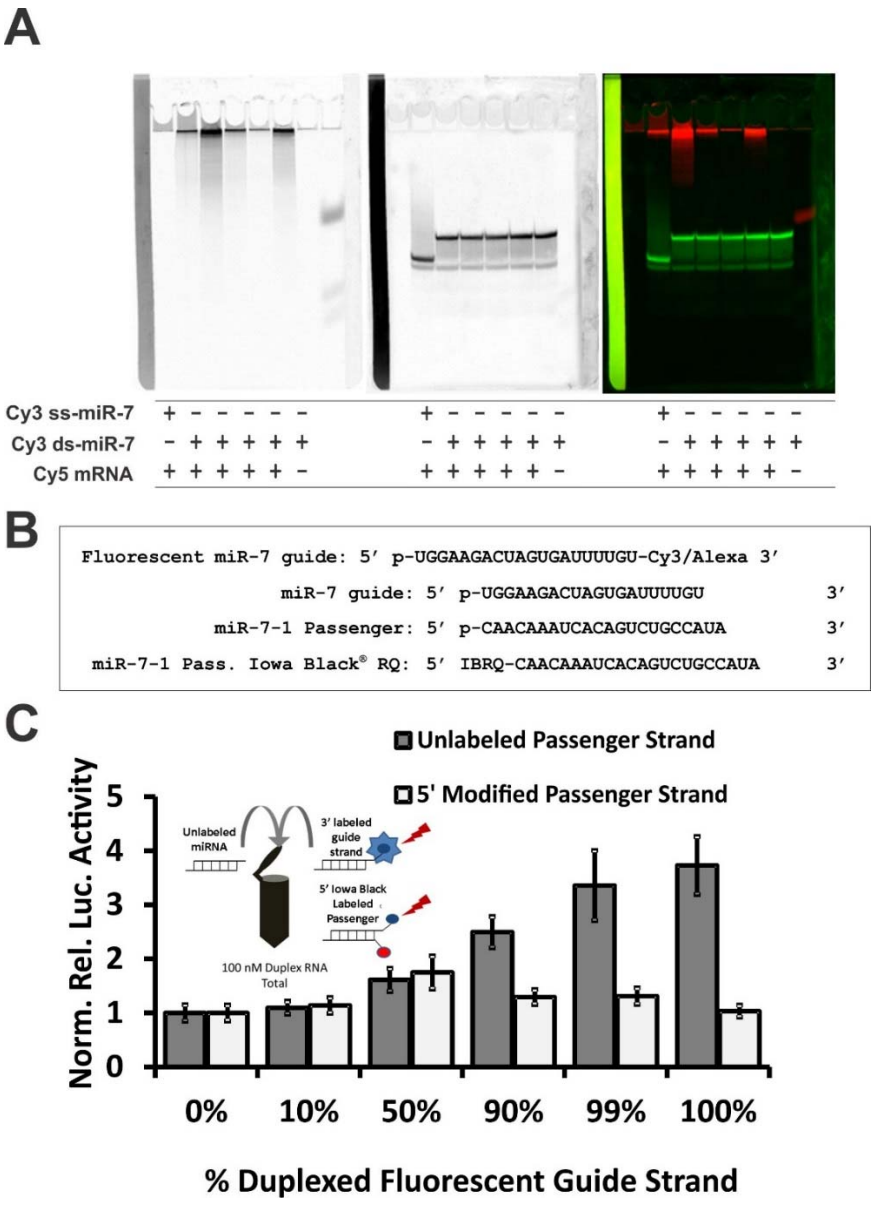

Figure S4. 3’ fluorescently labeled miRNA represses a luciferase reporter *in cellulo*. (A) Gel electrophoretic mobility shift assay of microinjection solutions containing 0.1 μM single-stranded (ss) or duplexed (ds) Cy3-miR-7 with varying concentrations of Cy5-body labeled mRNA. The 15% non-denaturing PAGE gel was scanned for Cy5 (red, left pane), Cy3 (green, middle pane), and the two images were overlaid (right pane). Cy5-body labeled mRNA was added to a concentration of, from left to right, 0.01 μM, 0.1 μM, 0.01 μM, 0.001 μM, 0.1 μM, and 0 μM. Gel shifting was observed in the form of smearing only when single-stranded miR-7 (ss-miR-7) guide strand was mixed with FLuc mRNA. (B) Labeling strategies and sequences of purchased miR-7 passenger and guide strand RNAs. (C) Luciferase response of dual luciferase reporter plasmid, with FLuc containing 11 miR-7 MRE sites, co-transfected in with 100 nM of total duplexed miR-7. Proportions of the 100 nM duplexed miR-7 were replaced with 3’ fluorescently labeled miR-7 containing either unlabeled passenger strand (dark grey bars) or 5’ Iowa Black® RQ labeled passenger strand. Dose dependent miR-7 repression was observed for labeled miR-7 duplexed with dark passenger strand. Duplexing the fluorescent miR-7 with 5’ Iowa Black® labeled passenger strand reversed this finding.

Oligonucleotide List

1 miR-7 Site

Forward	TCGAGTCTAGAGGTACCGGTTTTATTGTTATTCTGTCTTCCAGGAGCGGCCGCCCTGCAGGCATGCAAGCT
Reverse	AGCTTGCATGCCTGCAGGGCGGCCGCTCCTGGAAGACAGAATAACAATAAAACCGGTACCTCTAGACTCGA

2 miR-7 Sites

Forward	TCGAGTCTAGAGGTACCGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGAGCGGCCGCCCTGCAGGCATGCAAGCT
Reverse	AGCTTGCATGCCTGCAGGGCGGCCGCTCCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAATAAAACCGGTACCTCTAGACTCGA

3 miR-7 Sites

Forward #1	TCGAGTCTAGAGGTACCGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCT
Forward #2	TCCAGGTTTTATTGTTATTCTGTCTTCCAGGAGCGGCCGCCCTGCAGGCATGCAAGCT
Reverse #1	GAATAACAATAAAACCTGGAAGACAGAATAACAATAAAACCGGTACCTCTAGACTCGA
Reverse #2	AGCTTGCATGCCTGCAGGGCGGCCGCTCCTGGAAGACAGAATAACAATAAAACCTGGAAGACA

4 miR-7 Sites

Forward #1	TCGAGTCTAGAGGTACCGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGTTTTAT
Forward #2	TGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGAGCGGCCGCCCTGCAGGCATGCAAGCT
Reverse #1	AACCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAATAAAACCGGTACCTCTAGACTCGA
Reverse #2	AGCTTGCATGCCTGCAGGGCGGCCGCTCCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAATAA

5 miR-7 Sites

Forward #1	TCGAGTCTAGAGGTACCGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCT
Forward #2	TCCAGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGAGCGGCCGCCCTGCAGGCATGCAAGCT
Reverse #1	AGCTTGCATGCCTGCAGGGCGGCCGCTCCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAATAAAACCTGGAAGACA
Reverse #2	GAATAACAATAAAACCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAATAAAACCGGTACCTCTAGACTCGA

6 miR-7 Sites

Forward #1	TCGAGTCTAGAGGTACCGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTG TTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGTTTTAT
Forward #2	TGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGT TATTCTGTCTTCCAGGAGCGGCCGCCCTGCAGGCATGCAAGCT
Reverse #1	AGCTTGCATGCCTGCAGGGCGGCCGCTCCTGGAAGACAGAATAACAATAA AACCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAATAA
Reverse #2	AACCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAATAAA ACCTGGAAGACAGAATAACAATAAAACCGGTACCTCTAGACTCGA

11 miR-7 Sites

Forward #1	TCGAGTCTAGAGGTACCGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTG TTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTC
Forward #2	TTCCAGGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTC CAGGTTTTATTGTTATTCTGTCTTCCA
Forward #3	GGTTTTATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGT TTTATTGTTATTCTGTCTTCCAGGTTT
Forward #4	TATTGTTATTCTGTCTTCCAGGTTTTATTGTTATTCTGTCTTCCAGGAGCGG CCGCCCTGCAGGCATGCAAGCT
Reverse #1	AGCTTGCATGCCTGCAGGGCGGCCGCTCCTGGAAGACAGAATAACAATAA AACCTGGAAGACAGAATAACAATAAAACCTGGAAGAC
Reverse #2	AGAATAACAATAAAACCTGGAAGACAGAATAACAATAAAACCTGGAAGACA GAATAACAATAAAACCTGGAAGACAGAAT
Reverse #3	AACAATAAAACCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATA ACAATAAAACCTGGAAGACAGAATAACAA
Reverse #4	TAAAACCTGGAAGACAGAATAACAATAAAACCTGGAAGACAGAATAACAAT AAAACCGGTACCTCTAGACTCGA

References

1. Broderick JA, Salomon WE, Ryder SP, Aronin N, Zamore PD (2011) Argonaute protein identity and pairing geometry determine cooperativity in mammalian RNA silencing. RNA 17:1858-1869.
2. Agarwal V, Bell GW, Nam JW, Bartel DP (2015) Predicting effective microRNA target sites in mammalian mRNAs. Elife 4.