SUPPLEMENTARY DATA

Circularized synthetic oligodeoxynucleotides serve as promoterless RNA polymerase III templates for small RNA generation in human cells

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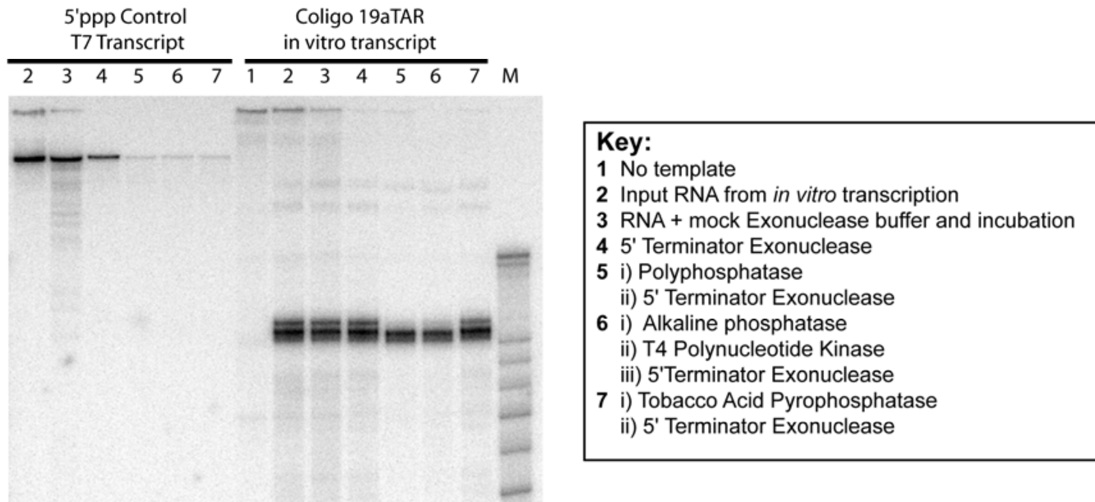
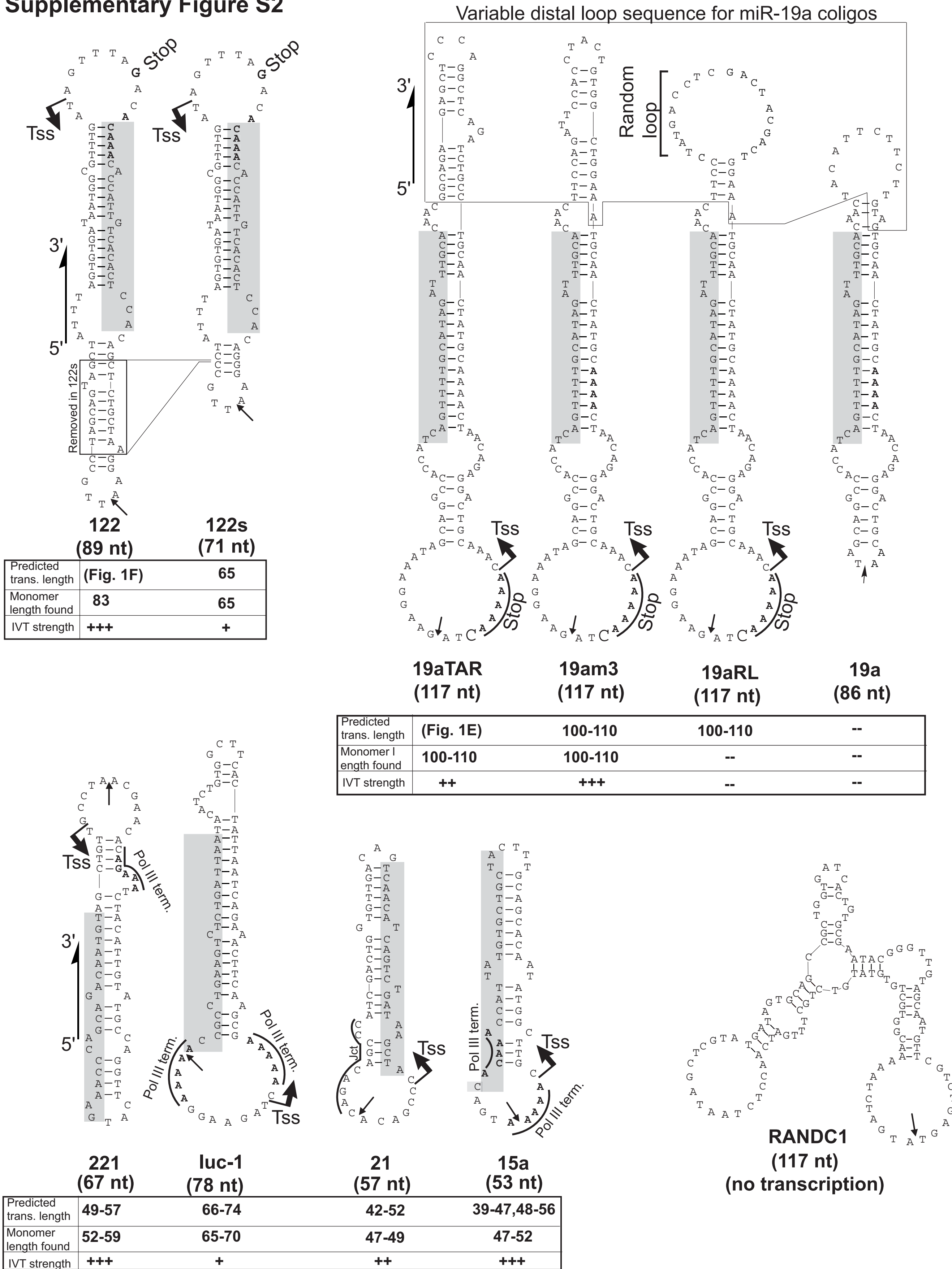
Supplementary Figure S1 5' End Characterization of Coligo 19aTAR Transcripts



Procedure: Positive control RNA was a T7 RNA polymerase *in vitro* transcript supplemented with total RNA extracted from HEK293T whole cell extract to normalize unlabeled non-specific RNA. Transcripts were Trizol extracted, ethanol precipitated, resuspended and treated sequentially for 60 min at 37 degrees C according to the **Key**, then separated on a 9% DPAGE.

Terminator 5' to 3' Exonuclease (Epicentre): degrades 5' mono phosphorylated RNA.
Polyphosphatase (Epicentre): removes polyphosphate, leaving 5' mono phosphate
Tobacco Acid Pyrophosphatase (Epicentre): Removes 5' cap, leaving 5' mono phosphate
Alkaline phosphatase (Promega): removes all phosphates to leave 5' OH
T4 polynucleotide kinase (New England Biolabs): puts 5' phosphate on unmodified 5' ends

Supplementary Figure S2



	122	122s
Predicted trans. length	(Fig. 1F)	65
Monomer length found	83	65
IVT strength	+++	+

	19aTAR	19am3	19aRL	19a
Predicted trans. length	(Fig. 1E)	100-110	100-110	--
Monomer l ength found	100-110	100-110	--	--
IVT strength	++	+++	--	--

	221	luc-1	21	15a
Predicted trans. length	49-57	66-74	42-52	39-47,48-56
Monomer length found	52-59	65-70	47-49	47-52
IVT strength	+++	+	++	+++

Supplementary Figure S2
Sequence and predicted secondary structure for all coligos used in this study. Numbers indicate the human miRNA on which the sequence is based. Transcription start and stop sites for coligo **122** and **19aTAR** were identified by sequencing data; all others are predicted based on similarity to **122** and **19aTAR** or on the size of the observed transcripts. In the case of coligo **21**, which has no RNAP III termination sequence, the predicted termination site is set as the 5' end of the stem-large loop junction. Shaded areas indicate cDNA of mature miRNA. Small arrows: circularization site.

Preparation of cytosolic and nuclear extracts for IVT. Different methods were used to obtain satisfactory cytosolic and nuclear fractionation. In order to obtain the highest cleanliness of each fraction (< 5% contamination of material from one fraction in the other) cytosolic and nuclear fractions were prepared from separate cell populations. These extracts were used for IVT in Figure 6F.

The nuclear fraction was prepared as follows: 3 x 10 cm dishes of confluent HEK293T were washed 3x with ice cold PBS and the dishes were placed on ice. 500μl of fractionation buffer (10 mM HEPES-KOH pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, 0.04% NP-40, Protease Inhibitor cocktail) were added to each 10 cm dish, the cells were scraped to one side of the dish and transferred with a 1 ml pipette tip to 15 ml conical tubes. Cells were incubated on ice for 10 min and spun at 4°C for 6 min at 2000 rpm. The supernatant was discarded; the nuclei were washed twice with cold 1 ml 0.5x fractionation buffer and pelleted in between washes as above. The pellet was resuspended in 200 μl fractionation buffer adjusted to 0.3 M NaCl, incubated on ice for 10 min with occasional vortexing. Nuclei were disrupted by dounce homogenization using a tight fitting, pre-chilled type A pestle for 20 strokes. The debris was spun down for 20 min, 13000 rpm at 4°C, the supernatant (nuclear fraction, 3 mg/ml total protein compared with BSA using Bradford assay) was transferred to a new pre-chilled tube and adjusted to 15% Glycerol. Aliquots were snap frozen in liquid nitrogen and stored at –80°C.

The cytosolic fraction was prepared as follows: initial steps were same as for the nuclear fraction but after resuspension in fractionation buffer the cells were monitored for lysis under a light microscope until around 50% of the cells had lysed. This reduced the total amount of protein but prevented significant contamination of the cytosolic fraction by any lysed nuclei. Sufficient lysis was observed in less than five minutes after which the fraction was spun down for 1 min at 13000 rpm at 4°C to pellet nuclei, the supernatant was transferred to another pre-chilled tube and spun for an additional 15 min at 13000 rpm at 4°C to pellet residual cellular debris. The supernatant after this spin, designated cytosolic extract, was adjusted to 15% Glycerol and 0.3 M NaCl to adjust the salt concentration to that of the nuclear fraction, (total protein was 5 mg/ml), snap-frozen in aliquots and stored at –80°C. Equal volumes of each extract were used for the transcription and Western blotting shown in Fig. 6F.

IVT using cytosolic and nuclear extracts with inhibitors: equal volumes (8 μl) of cytosolic (5 mg/ml total protein) and nuclear extracts (3 mg/ml total protein) were used in IVT (standard conditions) including inhibitors or solvent (as control, water or DMSO) in the reaction setup. We note that the inhibitory effect of ML-60218 in IVT reactions appeared to be sensitive to the buffer (or other condition) used for extract preparation, possibly causing less effective inhibition of transcription in IVT reactions, while it was consistently >90% in transfection experiments.

Cytosolic-Nuclear fractionation for coligo and transcript location. In order to directly compare cytosolic to nuclear fractions for experiments in Figures 6D and 6E, separations were carried out using Fermentas' ProteoJET™ kit (K0311) according to the manufacturer's instructions. Transfections were carried out in 6 cm cell culture dishes using 200 pmoles of coligo or linear form (final concentration of 40 nM) and 35 μl PolyFect. For transfection of isotopically labeled template, 5 pmoles of template labeling reaction product were transfected together with the unlabeled templates for 24 h before harvesting. Cells were harvested by trypsinization, washed

twice with cold PBS and the pellet was lysed with 10 packed cell volumes. The cells were lysed on ice for 5 min, the nuclei were spun down at 700 rcf for 5 min, the supernatant was transferred to a new pre-chilled 1.5 ml tube and the nuclei were washed twice with 500 µl nuclei wash buffer. The nuclei were resuspended in 240 µl nuclei storage buffer and lysed by addition of 10 µl nuclear lysis buffer, kept on ice for 10 min with intermittent vortexing. Both cytosolic and nuclear fractions were spun to collect debris for 20 min (4°C, 13000 rpm) the extract was transferred to a new pre-chilled tube and stored at –80°C.

Note on methods of cytosolic/nuclear fractionation: The effectiveness of separation using the ProteoJET™ kit varied from <10% to up to 30% nuclear leakage (i.e. contamination of the cytosolic fraction with nuclear proteins) in the lysis step. Similar results were obtained by using Ambion's PARIS™ kit (AM1921) according to the manufacturer's instructions. A method utilizing 40 µg/ml Digitonin in RSB-100 buffer (10 mM Tris-HCl, pH 7.4, 100mM NaCl, 2.5 mM $MgCl_2$) also resulted in a clean nuclear preparation but significant contamination of the cytosolic fraction with nuclear material, similar to the two kit-methods described above. (These were not used in any of the included figures, but did have coligo transcription activity similar to those used in the figures.) Overall, we note that cytosolic/nuclear fractionation using literature and kit methods is not as trivial as frequently depicted in the literature, and required repetitions and care to achieve the satisfactory separations used in the Figures.

Quantitative Northern blotting of *in vitro* transcription reactions

The plasmids used to generate the unlabeled RNA standards (p19aTAR or p122) were transcribed by T3 RNA polymerase in 100 µl using 2 µg of linearized plasmid and 50 units of T3 RNA polymerase (Promega) according to the T3 manufacturer's protocol. For Figure 3A (19aTAR), the plasmid-derived transcript was excised from a 6% DPAGE gel and eluted for 3 h at 37°C into RNA elution buffer (0.5 M NH_4OAc, 1 mM EDTA, 0.2% SDS). For Figure 3B (122), at the end of a 3 h IVT reaction using the linearized plasmid, 3 units of DNase I were added and the incubation was continued 30 min at 37°C, phenol/chloroform/isoamyl alcohol (PCI) extracted and ethanol precipitated. Quantification of RNA in both cases after resuspension was done by (1) absorbance at 260 nm (in 8 M urea) and (2) a comparison, on a Stains-All stained 9% DPAGE gel, with ss DNA oligonucleotides of known concentration. Radioactively labeled Northern probes used to detect transcript levels were generated by T7 RNAP (40 units) from linearized p122 and p19aTar and isolated from an excised gel slice. IVT was carried out for 1 h at 37°C in 40 mM Tris-HCl pH 7.9, 6 mM MgCl2, 10 mM DTT, 2 mM spermidine; 0.6 mM each ATP, CTP, GTP, 0.1 mM UTP, plus ~2 µCi [α-32P]s-UTP, 1unit/µl RNAse inhibitor, followed by PCI extraction and ethanol precipitation.

For RNA produced from coligo templates in HEK293T WCE IVT, RNA was extracted using 150 µl Trizol/20 µl IVT volume. The RNA was treated with DNase I (NEB) for 1 h, ethanol precipitated and blotted as described above. As loading control, the blot was re-hybridized to a 5' [γ-^{32}P]-ATP end-labeled DNA oligonucleotide (5'-TGGACCTTGAGAGCTTGTTTGGAGGTT) complementary to a portion of the endogenous 7SK RNA sequence or U2 snRNA, as indicated. 20 pmoles of end-labeled DNA oligonucleotide were used for hybridization overnight at 45°C. For Figure 3D, lanes 3 and 4, the 19aTAR coligo templates (L and C) were added to WCE and processed immediately as described above but without IVT incubation; for lanes 5 and 6, the

19aTAR coligo templates were electrophoresed as standards, and show the full Northern blot cross-reactive signal for the DNA templates if no DNase I treatment is used.

RNase protection assay (RPA). The probe was generated by [α-^{32}P]-UTP T7 IVT from p19aTAR transcription as described above, excised and eluted from 6% DPAGE, PCI extracted and ethanol precipitated. For hybridization, 1/20 of the eluted probe was precipitated together with 12 μg RNA prepared from linear or circular 19aTAR transfected HEK293T cells, or untransfected cells, and hybridized in 10 μl RPA hybridization buffer (Ambion AM1415) at 55°C overnight. RNA was then digested with an RNAse A/T1 cocktail in 150 μl RNase digestion buffer according to the manufacturer's instructions for 30 min at 37°C. The RNA was ethanol precipitated with 10 μg glycogen, and separated over 10% DPAGE. 1/20 of the undigested input probe (p- in Fig. 5C) or the probe digested without cellular RNA (p+ in Fig. 5C) were loaded for reference. The probe's T7 5' start site and 3' end are offset from the coligo transcript's 3' and 5' ends (judged by sequencing results shown in Fig. 1E), leading to a protected fragment that is smaller than 19aTAR's normal ~110 nt IVT transcript.

19aTAR RNA whole transcript cDNA sequencing

A typical coligo 19aTAR IVT was scaled up 10-fold (200 μl, 250 μg WCE, 100 nM coligo, no ^{32}P label). Total RNA was recovered using TriZol, resuspended in 150 μl annealing buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 6 mM $MgCl_2$). A 5' RACE experiment (see below) had shown that transcripts contained the intact TAR loop. To enrich the eluted fraction for the 19aTAR transcripts over the abundant endogenous HEK293T RNA, a biotin-streptavidin selection was carried out using a 5' Biotin labeled ss DNA oligonucleotide complementary to the TAR RNA sequence (5'Biotin-CGGCAGAGAGCTCCCAGGCTCAGATCTGCC-3'). Streptavidin-coated beads (Dynabeads MyOne Streptavidin C1, Invitrogen) were prepared as follows: to remove any RNAse contamination on the beads, 60 μl of 50% beads slurry were washed twice for 2 minutes each with 200 μl 100 mM NaOH/50 mM NaCl in DEPC treated ddH_2O. The beads were then washed 3X with 500 μl 100 mM NaCl and resuspended in 150 μl 2X binding/washing buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 2 M NaCl). The RNA solution was added to 50 pmol of the 5'Bio-oligo, heated to 93°C for 4 min, and allowed to cool to room temperature. The nucleic acids were added to the previously washed beads in 2X binding buffer to produce the 1X binding medium. The mix was incubated at room temperature with gentle rocking for 30 min. The beads were magnetically concentrated, the supernatant removed, and the beads were washed 5X with 500 μl 1X binding buffer. To elute the selected RNA from the beads, the reaction was PCI extracted. The aqueous phase was ethanol precipitated with 10 μg glycogen. The selected RNA was purified using 6% DPAGE. The gel region between 90-130 nt was excised and eluted overnight in 800 μl gel elution buffer at 37°C. The eluted RNA was PCI extracted, ethanol precipitated with glycogen and resuspended in ddH_2O. The 3' adaptor ligation was performed without ATP using 900 ng pre-adenylated Universal miRNA Cloning Linker (NEB) and 100 units of truncated T4 RNA ligase 2 (NEB) in T4 RNA ligase buffer with 12% PEG 8000, in a total volume of 20 μl for 2 h at 37°C. To remove unreacted adaptor, the reaction was PCI extracted, ethanol precipitated, resolved using 6% DPAGE and the gel slice between 80-150 nt was eluted as described above. Two units of Calf Alkaline Phosphatase (CIP, Promega) were used in case the RNA has a triphosphate at the 5' end. The RNA was PCI extracted, ethanol precipitated and resuspended in water. The 5' end was phosphorylated using ATP and T4

PNK. The 5’ adaptor ligation was carried out using Ambion’s RLM-RACE kit according to the manufacturer’s instructions. Briefly, 300 ng of the 5’ RNA adaptor were ligated to the eluted, 3’ adaptor-containing RNA using T4 RNA ligase 1 in T4 RNA ligase buffer. 3 μl of this reaction were used in a reverse transcription reaction with 30 pmol of MiRclon3NotI oligonucleotide for 1 h at 55°C using SuperScript III Reverse Transcriptase (Invitrogen). The reaction was stopped by heating to 75°C for 15 min. 1 unit RNAse H was added for 30 min at 37°C and 1 μl of this reaction was used for nested PCR amplification using the 5’ outer primer and MiRclon3NotI reverse primer for 30 cycles. Inner PCR was performed with the 5’ inner primer and MiRclon3NotI primer for 35 cycles. The resulting PCR product was purified on 2% agarose; the band around 200 bp was excised and eluted using an agarose gel extraction kit (GenScript). The eluted PCR product was digested with the restriction enzymes. The digested PCR was again gel-purified, eluted, recovered and ligated into pBluescript II SK previously digested with the same restriction enzymes and gel purified at a molar ratio of 3:1 (insert:vector) at 16°C overnight using T4 DNA ligase with 1 mM final ATP in a 20 μl reaction. 5 μl of the ligation were transformed into 75 μl competent *E. coli* and the bacteria were plated onto LB-Amp plates supplemented with 40 μl each of 20 mg/ml X-Gal and 100 mM IPTG to allow for blue-white selection. White colonies were grown and miniprep plasmids were analyzed for inserts using the same restriction enzymes used for subcloning the insert. 34 insert-containing clones were picked and sequenced by Macrogen; 13 contained inserts originating from 19aTAR transcripts (Fig. 1E), the rest contained ribosomal RNA sequences.

Preliminary 19aTAR RLM-RACE 5’ end sequencing. RNA was generated in a 10-fold scale up of an unlabeled IVT reaction. The gel slice between 80-150 nt from a 6% DPAGE was excised and eluted overnight in elution buffer. The RNA was treated with CIP, 5’ phosphorylated with PNK and joined to the 5’ adaptor as described above. Reverse transcription was performed using random decamers, followed by PCR using the 5’outer primer and a 19aTAR transcript-specific primer (19aT-fwd see below) for 30 cycles. 1 μl of this reaction was used in a nested PCR with primers 5’inner primer and 19aT-fwd (see below). The PCR products were separated on 2% agarose, the product at ~150 bp was eluted, digested with BamHI and cloned into pBluescript II SK as described above. Out of 15 clones, 8 began with the Tss site shown in Figure 1E. (A second start site was found beginning at the dC in the bulge loop 14 nt on the 5’ side of the Tss. This start site was not found in the full length sequencing procedure and thus remains unconfirmed, though it too is an A-rich ss loop.) In combination with the length of the IVT transcripts, the 5’ RACE allowed us to determine that the TAR loop was present in the full length transcripts represented by the 5’ RACE hits, and this fact was used in the biotin selection described above.

122 RLM-RACE 5’ end sequencing

In an effort to reduce the amount of cellular RNA for the 122 RLM-RACE end sequencing protocol, we first treated the HEK293T whole cell extract (WCE) with DEAE25 ion exchange resin: DEAE-sephadex A-25 beads (Amersham Biosciences) were washed five times with lysis buffer and 100 μl of a 50% suspension of beads was added to 500 μl WCE. The suspension was gently rotated for 30 min at 4°C and the procedure repeated using a new aliquot of DEAE beads. This procedure removed around 80% of short RNA as judged by Stains-All DPAGE visualization. This WCE was used in IVT of coligo **122** for 5’ RACE-RLM. Two RLM-RACE procedures were

used. First procedure: 250 μg protein from a DEAE-treated WCE was used in a 200 μl, [α-^{32}P]-UTP labeled IVT with 100 nM coligo 122. The extracted RNA was resolved using 6% DPAGE and the gel was exposed to film for 2 h. The aligned film was used to locate and excise the monomer transcript (~83 nt) which was eluted overnight in gel elution buffer at 37°C. To trim a possible 5' triphosphate to 5' monophosphate, the RNA was treated with 20 u of RNA 5' polyphosphatase (Epicentre). The 5' RACE adaptor ligation was done as described above and resuspended in 25 μl. 3 μl were used for reverse transcription with random decamers at 50°C for 1 h with the MMLV RT from Ambion's RLM-RACE kit in 25 μl. 1 μl was then used in outer PCR for 30 cycles with 5' outer primer and 5end122-6. Inner PCR was performed with 5' inner primer and 5end122-5 for 35 cycles. The PCR products between 60 and 100 bp were isolated from a 3% agarose gel and 1/10 of the eluted product was re-amplified for 30 cycles using the same primers as for inner PCR. The reaction was digested with BamHI and NotI, re-purified over a 3% agarose gel and the eluted fragment was quantitated by UV absorbance at 260 nm. Cloning was performed in a 3:1 (insert:vector) ratio as described above. Plasmids were purified from 3 ml overnight culture in LB-Amp and ~7μg plasmid were digested and examined by 10% native PAGE to check for inserts. Positive clones were sequenced by Macrogen. Second procedure: Performed as above except that the coligo transcript-specific primer was located farther downstream of the transcription start site as determined by the first RACE experiment. Briefly, 1μl of the RT reaction (1/25th) was amplified with 5' inner primer and 5end122-2, the band around 100 bp was eluted from a 3% agarose gel and 1/10 of the eluted product was re-amplified using the same primers. The resulting product was digested with BamHI and NotI and cloned into pBluescript II SK as described above. Similar results were found in both experiments. 30 out of 30 positive clones contained 122 transcript cDNA and 27 are depicted in Figure 1F. Three related clones began 4 nt away at the -TTT- sequence in the stem and contained either an additional -a- or -acac- at the 5' end, which we assume to be artifacts of the sequencing procedure.

122 RLM-RACE 3' end sequencing

The RNA from a 10-fold scale up coligo 122 IVT reaction containing [α-^{32}P]-UTP was extracted with TriReagent, isopropanol precipitated and separated by 10% DPAGE. The single band at ~83 nt was excised and eluted overnight in elution buffer. The RNA was PCI extracted and precipitated as above. A poly(A) tail was added to the 3' end using 5 u *E. coli* poly(A) polymerase (NEB, #M0276S) in a 20 μl reaction plus 1 mM ATP for 1.5 h at 37°C. The RNA was PCI extracted, precipitated, and used as the template for reverse transcription using Ambion's 3'RACE adapter and ThermoScript Reverse Transcriptase at 55°C for 1 h. Sequence specific PCR was carried out using the 3'RACE Inner primer and 122aE3end2 in a 100 μl reaction for 35 cycles. The resulting PCR product was PCI extracted, ethanol precipitated, digested with 20 units BamHI, extracted, precipitated and separated over a 3% agarose gel. The band ~150 bp was isolated and ligated at a 3:1 (insert:vector) ratio into BamHI-cut and Calf Alkaline Phosphatase treated pBluescript II SK as described above. 80 μl competent *E. coli* cells were transformed with 6 μl ligation mix, grown on LB-Amp plates and white colonies were picked for plasmid miniprep. 19 out of the 19 positive clones were found to carry an insert originating from the 122 transcript (Fig. 1F). Although the RNA sequences for the coligo 122 single round transcripts shown in Figure 1 are described as "composite," the 3' RLM-RACE procedure actually sequenced up to the third nucleotide from the 5' end of the dominant transcript (found in 23 clones), or 98% of the entire transcript. This resulted from our

choice of the primer binding site on the reverse transcriptase reaction product, i.e. primer 122aE3end2 (see below.) The 5' RLM-RACE was required to know the extreme 5' ends. Lastly, four 3' RACE clones ended with UGU, as indicated in Figure 1F, but had one or two untemplated, non-A nt between the templated 3' end and the added poly(A) tail, which we assume to be an artifact of the sequencing procedure, and are not counted in Fig. 1F.

Primers used in sequencing procedures. Universal miRNA Cloning Linker (NEB) 5'-rAppCTGTAGGCACCATCAAT–NH2-3', 5'adaptor (Ambion RLM-RACE kit) 5'-GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA; MiRclon3NotI (IDT) 5'-AGCGGCCGCCTGCAGATTGATGGTGCCTACAG; 5' outer primer (Ambion RLM-RACE kit) 5'-GCTGATGGCGATGAATGAACACTG; 5' inner primer (Ambion RLM-RACE kit) 5'-CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG; 5end122-5 (IDT) 5'-CAGCGGCCGCGAATTCTATTTAGTGTGATAA; 5end122-6, 5'-AAGCGGCCGCGAA-TTCTTGCCTAGCAGTAGC; 5end122-2 (IDT) 5'-GTGCGGCCGCGAATTCATTGT-CACACTCCA; 19aT-fwd (IDT), 5'- GCATGGATCCGAAGGAAATAGCAGGCCA; 3' RACE adapter: 5'-GCGAGCACAGAATTAATACGACTCACTATAGGT12(A,G or C)N-3';

3' RACE Inner Primer: 5'-CGCGGATCCGAATTAATACGACTCACTATAGG-3'.

122aE3end2: 5'-TAGGATCCTCACAAACGCCATTATCACACT-3'.

Production of isotopically labeled coligo (Figure 5A, 6D). 100 pmoles of 5'phosphate containing coligo **19aTAR** were treated with 3 units calf alkaline phosphatase for 1 h at 37°C to remove the 5' phosphate. After phenol/chloroform extraction and ethanol precipitation, the oligonucleotide was [γ-^{32}P]-ATP end-labeled with 20 units PNK enzyme and 2 µl [γ-^{32}P]-ATP (6000 Ci/mmol) and incubated for 1 h at 37°C. The oligonucleotide was again extracted and precipitated. 75% of the end-labeled oligonucleotide was circularized as described previously (1). The circular and unreacted linear forms were separated over 7% DPAGE and eluted overnight in 0.3 M NaCl at room temperature. After elution, the oligonucleotides were phenol/chloroform extracted, ethanol precipitated and resuspended in 10 µl TE buffer.

Western blotting. 10% SDS-PAGE (7% for RNA polymerase III large subunit RPC2) was used for electrophoresis of nuclear and cytosolic fractions (same volume as used for IVT reaction shown in same panel) and blotted on nitrocellulose membrane. Blots were blocked for 1 h in 3% non-fat dry milk in PBS. Incubation with antibodies was performed in PBS+0.1% Tween-20 (PBST). Primary antibodies were incubated over night at 4°C, secondary antibodies for 2 h at room temperature. Primary antibodies were purchased from Novus Biologicals (CSTF3, H00001479-M01), Santa Cruz Biotechnology (β-tubulin sc-80011), Bethyl (H4 histone A300-646A and RPC2, A301-855A, subunit of RNAPIII). Secondary antibodies were from Calbiochem (goat anti-rabbit IgG Peroxidase Conjugate DC03L) and Santa Cruz Biotechnology (goat anti-mouse IgG_1-HRP, sc-2060). Dilutions of primary and secondary antibodies were made according to the manufacturer's instructions. Blots were developed using either the ECL system, or ECL Plus with Phosphorimager scanning (GE Healthcare).

Linear precursor sequences for coligos

19a:

5’pTAGCAGGCCACCATCAGTTTTGCATAGATTTGCACAACTACATTCTTCTTGTAGTGCAACTATGCAAAACT AACAGAGGACTGCAA

19aTAR:

5’pGAAGGAAATAGCAGGCCACCATCAGTTTTGCATAGATTTGCACAACGGCAGAGAGCTCCCAGGCTCAGA TCTGCCTGCAACTATGCAAAACTAACAGAGGACTGCAAACAAAAACTA

122:

5’pTTGCCTAGCAGTAGCTATTTAGTGTGATAATGGCGTTTGATAGTTTAGACACAAACACCATTGTCACACT CCACAGCTCTGCTAAGGAA

122TAR:

5’pAGGAAATAGCCTAGCAGTAGCTATTTAGTGTGATAATGGCGTTTGATAGGGCAGAGAGCTCCCAGGCTC AGATCTGCCCACAAACACCATTGTCACACTCCACAGCTCTGCTAAGGAAACAAAA

122s:

5'pTTGCCCTATTTAGTGTGATAATGGCGTTTGATAGTTTAGACACAAACACCATTGTCACACTCCACAGGGA A

RANDC1:

5’pATGATCTAAAAACGGTGCTGTGTATGTCTGCTTTGATCAACCTCTAATAGCTCGTATGATAGTGCAGCCG CTGGTGATCACTGTGCGAATACGGGTTGTAGCAATGTTCGTCTGAGT

19aRL:

5’pGAAGGAAATAGCAGGCCACCATCAGTTTTGCATAGATTTGCACAACTTCCCTATGACCTCGACTACGACT GGAAATGCAACTATGCAAAACTAACAGAGGACTGCAAACAAAAACTA

221:

5’pACGAACACAGAAATCTACATTGTATGCCAGGTTCATGAAACCCAGCAGACAATGTAGCTGTTGCCTA

15a 5’pAATCCACAAACCATTATGTGCTGCTACTTTGCAGCACAATATGGCCTGCACAA

21: 5’pCAGACAGCCCATCGACTGGTGTTGACAGTCAACATCAGTCTGATAAGCTACCCGACA

SUPPLEMENTARY REFERENCE

1. Seidl, C.I. and Ryan, K. (2011) Circular single-stranded synthetic DNA delivery vectors for microRNA. *PLoS One*, **6**, e16925.