Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A Cas12a-mediated cascade amplification method for microRNA detection

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Experimental sections

Ligation-mediated PCR

The ligation reaction was carried out with a 10 μL reaction mixture containing 200 nM ligation substrate A, 200 nM ligation substrate B-1, 8 U Ribonuclease inhibitor, 1× T4 RNA ligase 2 reaction buffer, 5 U T4 RNA ligase 2, and 20 nM let-7a. Firstly, ligation substrate A, ligation substrate B-1, and let-7a were mixed and then heated at 65 °C for 3 min and 25 °C for 30 min to perform the hybridization. Following, the ligase buffer and T4 RNA ligase 2 were added in the mixture, and the reaction mixture was incubated at 37 °C for 60 min to complete the ligation reaction. After the ligation reaction, the products were immediately put on ice. A volume of 2 μL of the ligation product was transferred to PCR reaction mixture with final volume of 20 μL. The PCR reaction mixture included 0.4 μL of Phanta Max Super-Fidelity DNA Polymerase, 0.4 μL of 10 μM F-primer, 0.4 μL of 10 μM R-primer, 0.4 μL of 10 mM dNTP mix, 10 μL of 2× Phanta Max buffer, and 6.4 μL of DEPC H_2O. The PCR reaction was carried out using the following protocols: hot start of 95 °C for 5 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 10 s, finally an extension at 72 °C for 10 min.

The RNA cleavage reaction of 8-17E DNAzyme

The RNA cleavage reaction was carried out with a 10 μL reaction mixture containing 500 nM 8-17E DNAzyme, 500 nM 8-17E substrate, 1× annealing buffer, and divalent cation. Firstly, 8-17E DNAzyme and 8-17E substrate were mixed and then heated at 65 °C for 3 min and 25 °C for 30 min to perform the hybridization. Following, the divalent cation was added in the mixture, and the reaction mixture was incubated at 37 °C for 60 min to complete the RNA cleavage reaction.

In vitro transcription and purification of crRNAs

The transcription templates were prepared through annealing crRNA template-1 to crRNA template-2 in 1× annealing buffer. crRNAs were synthesized using a HiScribe T7 High Yield RNA Synthesis Kit and the reaction was performed at 37 °C overnight (about 12 h). The transcribed crRNAs were purified by a HiPure RNA Pure Micro Kit, redissolved in DEPC H_2O, and stored at -80 °C for further use. The concentrations of the prepared crRNAs were measured by a NanoDrop 2000 UV−vis spectrophotometer.

Cas12a-mediated cleavage reaction

The substrates of Cas12a *trans*-cleavage reaction were prepared by annealing 5-fold molar excess of the activator-1 to activator-2 in 1× annealing buffer heating at 95 °C for 3 min and slow-cooling to 25 °C. The cleavage experiment was carried out in 20 μL reaction mixture containing 50 nM Cas12a, 100 nM crRNA, 0.1 U RNase inhibitor, 10 nM activator, and 50 nM reporter in 1× NEBuffer 2.1. First, Cas12a-crRNA complex was preassembled by incubating 50 nM Cas12a and 100 nM crRNA for 30 min at 37 °C in 1× NEBuffer 2.1. Then, 10 nM activator, 50 nM reporter, and 0.1 U RNase inhibitor were added to the Cas12a-crRNA complex solution with a final volume of 20 μL. The above mixture was incubated at 37 °C for 60 min, and then 1 μg Proteinase K was added to terminate the reaction.

qRT-PCR analysis of miRNA

The cDNA samples were prepared using Mir-X miRNA First-Strand Synthesis Kit. PCR reaction mixture was prepared in a volume of 20 μL, consisting of 2 μL of cDNA sample, 10 μL of 2× SYBR qPCR SuperMix Plus, 0.5 μL of 10 μM let-7a-primer, 0.5 μL of 10 μM mRQ 3' primer, and 7 μL of DEPC H_2O. The PCR reaction was carried out using the following protocols: hot start of 95 °C for 5 min, followed by 45 cycles of 95 °C for 10 s, 50 °C for 30 s, and 72 °C for 20 s.

Supporting tables

Table S1. Sequence information of oligonucleotides used in this work.

Name	*Sequence (5'-3')
ligation substrate A	GGTCGAGCTGGACGGCGACGATCTACACTTAGTAGAAATTACTAACTATACArArC
ligation substrate B-1	PO_4-CTACTACCTCACCCTATAGTGAGTCGTATTAGTGATC
ligation substrate B-2	GATCACTAATACGACTCACTATAGGG
8-17E DNAzyme	TAGTAGAAATTATCCGAGCCGGTCGAAAACTATACA
8-17E substrate	TGAGGTAGTAGGTTGTATAGTTrAGTAATTTCTACTAAGTGTAGATC
activator-1	GCTTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGC
activator-2	GCCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGC
reporter	FAM-TTATT-BHQ1
crRNA template-1	GGTCGAGCTGGACGGCGACGATCTACACTTAGTAGAAATTACCCTATAGTGAGTCGTATTAATTTC
crRNA template-2	GAAATTAATACGACTCACTATAGGG
F-primer	GGTCGAGCTGGACGGC
R-primer	GATCACTAATACGACTCACTATAGGGTG
let-7a-primer	CGGTGAGGTAGTAGGTTGTATAGTT
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU
miR-21	UAGCUUAUCAGACUGAUGUUGA

miR-122	UGGAGUGUGACAAUGGUGUUUG

*****The catalytic core of 8-17E DNAzyme are in green font. PAM sequences are in blue font. The point mismatch bases in let-7d, let-7e, let-7i sequences compared to let-7a are in red font.

Table S2. Comparison with other methods for miRNA detection.

Method	LOD	Linear range	Reference
Spinach-based fluorescent light-up biosensors	3 pM	10 pM-10 nM	1
MB with duplex-specific nuclease amplification	3.8 pM	5 pM-500 pM	2
G-triplex based MB	0.25 pM	1 pM-5 nM	3
Amplified tandem spinach-based aptamer transcription	67.3 fM	100 fM-50 pM	4
RCA-CRISPR-split-HRP	100 fM	No test	5
DNA polymerase coupled with nicking endonuclease	1 fM	1 fM-100 nM	6
Quadratic isothermal amplification	10 fM	No test	7
This work	21.9 fM	100 fM-1 nM	/

Supporting figures

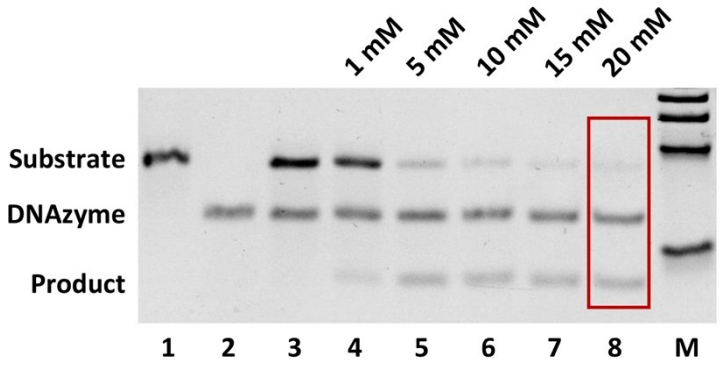

Fig. S1. Investigation on the influence of the concentration of Mg^{2+} on 8-17E substrate cleavage reaction. Lane 1: 500 nM 8-17E substrate. Lane 2: 500 nM 8-17E DNAzyme. Lanes 3-8: the reaction mixtures (500 nM 8-17E substrate and 500 nM 8-17E DNAzyme) in the presence of 0 mM, 1 mM, 5 mM, 10 mM, 15 mM, or 20 mM Mg^{2+}, respectively.

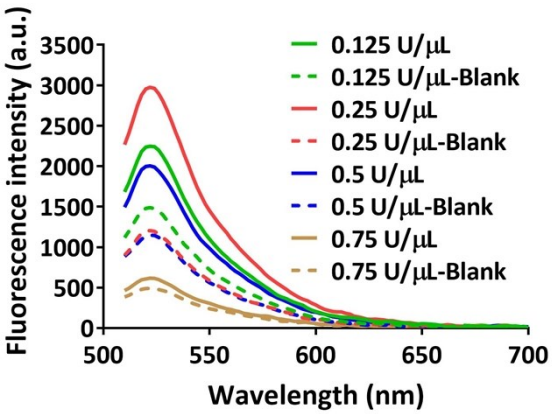

Fig. S2. Optimization of the concentration of T4 RNA ligase 2 in the presence of 10 nM let-7a. Fluorescence emission spectra responses to T4 RNA ligase 2 at different concentrations (0.125 U/μL, 0.25 U/μL, 0.5 U/μL, and 0.75 U/μL) in the absence and the presence of let-7a.

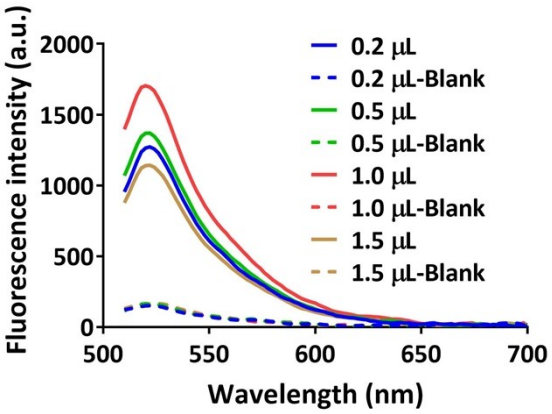

Fig. S3. Optimization of the amount of T7 RNA polymerase mix in the presence of 10 nM let-7a. Fluorescence emission spectra responses to T7 RNA polymerase mix at different amounts (0.2 μL, 0.5 μL, 1.0 μL, and 1.5 μL) in the absence and the presence of let-7a.

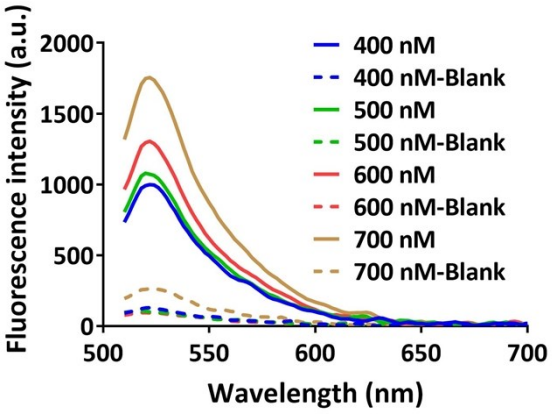

Fig. S4. Optimization of the concentration of 8-17E DNAzyme in the presence of 10 nM let-7a. Fluorescence emission spectra responses to 8-17E DNAzyme at different concentrations (400 nM, 500 nM, 600 nM, and 700 nM) in the absence and the presence of let-7a.

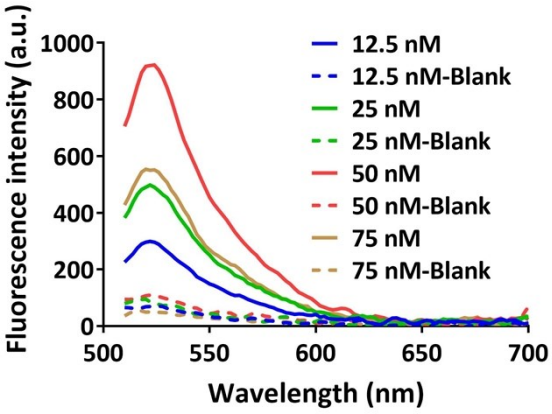

Fig. S5. Optimization of the concentration of Cas12a in the presence of 10 nM let-7a. Fluorescence emission spectra responses to Cas12a at different concentrations (12.5 nM, 25 nM, 50 nM, and 75 nM) in the absence and the presence of let-7a.

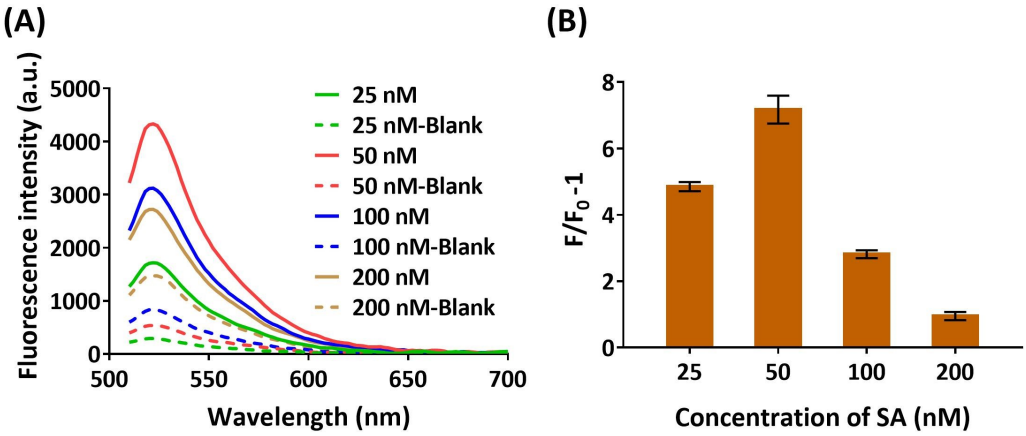

Fig. S6. Optimization of the concentration of ligation substrate A (SA) in the presence of 10 nM let-7a. (A) Fluorescence emission spectra responses to ligation substrate A at different concentrations (25 nM, 50 nM, 100 nM, and 200 nM) in the absence and the presence of let-7a. (B) Bar representing fluorescence ratio (F/F_0-1) responses in the presence of ligation substrate A at different concentrations. F and F_0 are the fluorescence intensities at a peak value of 518 nm in the presence and the absence of let-7a, respectively. The error bars represent the standard deviations of three repetitive measurements.

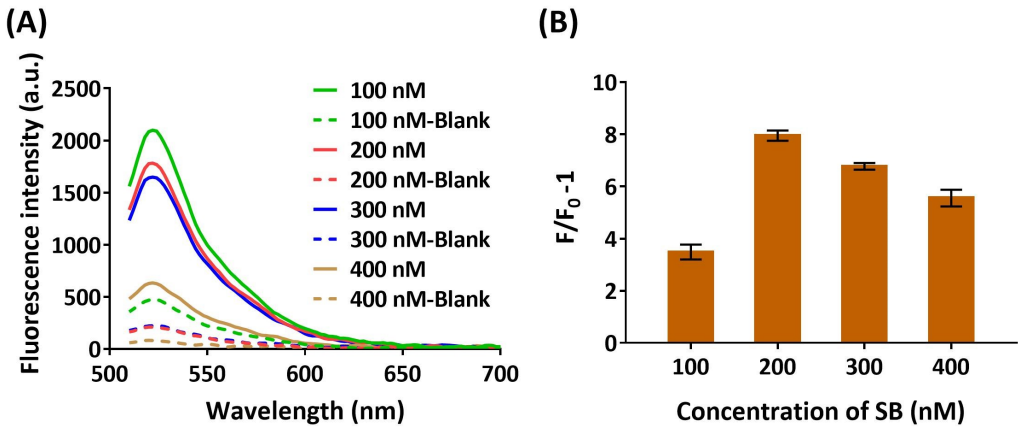

Fig. S7. Optimization of the concentration of ligation substrate B (SB) in the presence of 10 nM let-7a. (A) Fluorescence emission spectra responses to ligation substrate B at different concentrations (100 nM, 200 nM, 300 nM, and 400 nM) in the absence and the presence of let-7a. (B) Bar representing fluorescence ratio (F/F_0-1) responses in the presence of ligation substrate B at different concentrations. F and F_0 are the fluorescence intensities at a peak value of 518 nm in the presence and the absence of let-7a, respectively. The error bars represent the standard deviations of three repetitive measurements.

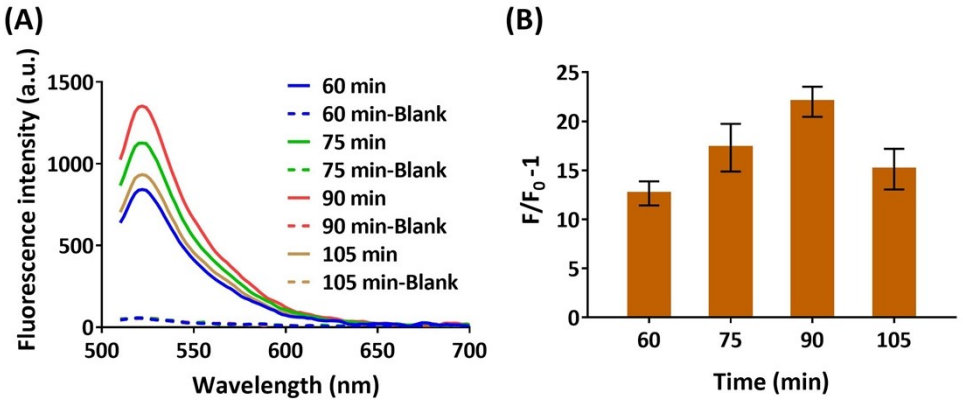

Fig. S8. Optimization of the reaction time of the second and third steps in the presence of 10 nM let-7a. (A) Fluorescence emission spectra responses to different reaction times (60 min, 75 min, 90 min, and 105 min) in the absence and the presence of let-7a. (B) Bar representing fluorescence ratio (F/F_0-1) responses to different reaction times. F and F_0 are the fluorescence intensities at a peak value of 518 nm in the presence and the absence of let-7a, respectively. The error bars represent the standard deviations of three repetitive measurements.

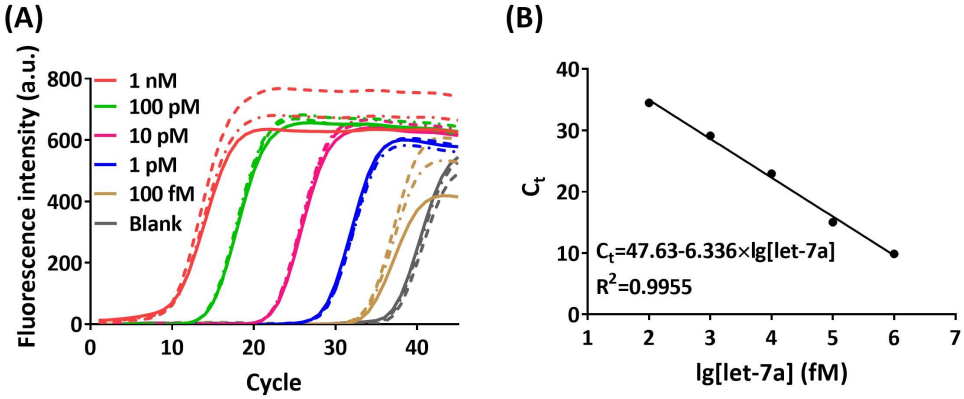

Fig. S9. (A) Real-time fluorescence detection of standard sample let-7a by qRT-PCR. (B) Linear relationship between the C_t and the logarithm of the concentrations of let-7a (100 fM, 1 pM, 10 pM, 100 pM, and 1 nM). The error bars represent the standard deviations of three repetitive measurements.

References

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