Supporting Information

CRISPR/Cas12a Assisted Ligation-Initiated Loop-Mediated Isothermal Amplification (CAL-LAMP) for Highly Specific Detection of microRNAs

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1.Table S1. Sequences of miRNAs and oligonucleotides used in this work.

Table S1. Sequences of miRNAs and oligonucleotides used in this work.

Name	Sequence(5′-3′ direction)
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUAUAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-143	UGAGAUGAAGCACUGUAGCUC
miR-205	UCCUUCAUUCCACCGGAGUCUG
$SLP\text{-}PAM_{let\text{-}7a}$	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGATTTTCCTCTGCT GTCGTTTGAACTATACAAC
$SLP_{let\text{-}7a}$	/P/CTACTACCTCATTTATCGTCGTGACTGTTTGTAATAGGACAGAGCCCCG CACTTTCAGTCACGACGAT
$SLP\text{-}PAM_{miR\text{-}21}$	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGATTTTCCTCTGCT GTCGTTTGTCAACATCAGT
$SLP_{miR\text{-}21}$	/P/CTGATAAGCTATTTATCGTCGTGACTGTTTGTAATAGGACAGAGCCCCGC ACTTTCAGTCACGACGAT
FIP	ATCGTCGTGACTGAAAGTGCGGGGCTCTGTCCTATTAC
BIP	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGA
$crRNA_{let\text{-}7a}$	GAAAUUAAUACGACUCACUAUAGGGAAUUUCUACUGUUGUAGAUAACU AUACAACCUACUACCUCAUU
$crRNA_{miR\text{-}21}$	GAAAUUAAUACGACUCACUAUAGGGAAUUUCUACUGUUGUAGAUUAGC UUAUCAGACUGAUGUUGAUU
Reporter	FAM-TTATT-BHQ1

Notes: The letter /P/ indicates the PO_4 modification at the 5' end.

2. Influence of reaction temperature of LAMP.

The reaction temperature of LAMP not only affects the hybridization efficiency of primers (FIP and BIP) but also affects the activity of *Bst* 2.0 WarmStart DNA polymerase. The influence of reaction temperature of LAMP for the CAL-LAMP-based miRNA assay was investigated by simultaneously detecting the blank, 100 aM, 1 fM, 10 fM, and 100 fM let-7a at different temperature of LAMP. As result shown in Figure S1 (d), when the LAMP was performed at 70 °C (d), almost no detectable fluorescence signals could be observed, indicating that LAMP efficiency was very low at 70 °C. It should be reasonable that the stem-loop in SLP and SLP-PAM and the hybridization between FIP/BIP and template are unstable. When the temperature of LAMP reduced to 55 °C (Figure S1 (a)), the fluorescence signals produced by 100 aM and 1 fM let-7a cannot be separated from blank and control indicating low sensitivity, which means that the activity of *Bst* 2.0 DNA polymerase would decrease under lower temperature. As demonstrated in Fig. S1 (b) and 1(c), when the temperature of LAMP was 60 °C or 65 °C, the fluorescence signals produced by different concentrations of let-7a obviously distinguished blank, control and each other. In this work, we selected 65 °C as the temperature of LAMP amplification.

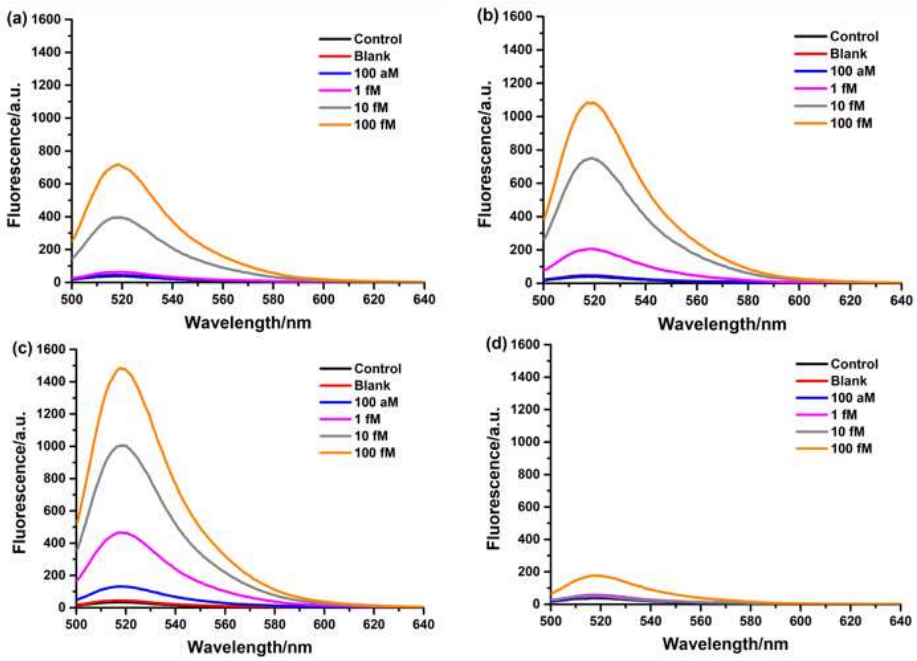

Figure S1. Effect of the temperature of LAMP for miRNA detection. The temperature of the LAMP was 55 ℃ (a), 60 ℃ (b), 65 ℃ (c) and 70 ℃ (d), respectively.

3. Effect of the temperature of Cas12a cleavage.

The reaction temperature of Cas12a cleavage affects the activity of Cas12a protein. To evaluate the effect of the temperature of Cas12a cleavage on miRNA detection, the CAL-LAMP was carried out according to the procedure in the experimental section except for the temperature of the Cas12a cleavage reaction. 100 aM, 1 fM, 10 fM and 100 fM let-7a were determined under 16 ℃, 25 ℃, 37 ℃ and 48 ℃ for Cas12a cleavage reaction, respectively. As shown in Figure S2, the temperature of the Cas12a cleavage reaction affected the intensity of the fluorescence signal. When the temperatures are 16 ℃ and 25 ℃, the fluorescence signal is low and the low-concentration let-7a cannot be distinguished from blank and control, which indicates low sensitivity. In contrast, when the temperatures are 37 ℃ and 48 ℃, the fluorescence signal produced by different concentration let-7a is higher than that of 16 ℃ and 25 ℃ and can be clearly distinguished from each other, which indicates high sensitivity. So we selected 37 ℃ for CAL-LAMP-based miRNA assay.

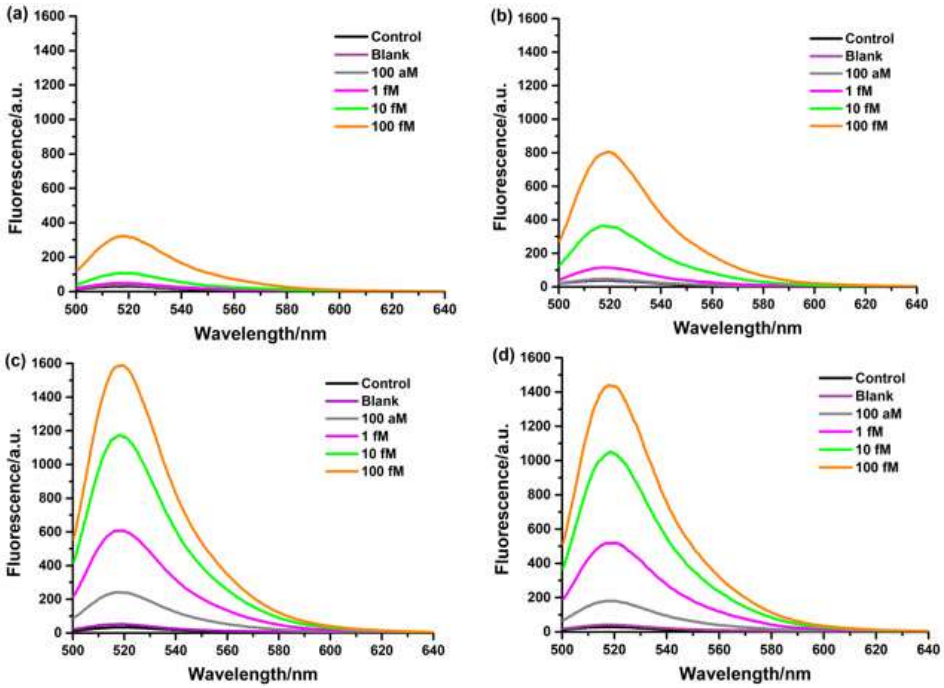

Figure S2. Effect of the temperature of Cas12a cleavage on miRNA detection. The temperature of the Cas12a cleavage was 16 ℃ (a), 25 ℃ (b), 37 ℃ (c) and 48 ℃ (d), respectively.

4. Effect of the reaction time of Cas12a cleavage.

To achieve the optimized conditions for the CAL-LAMP assay, we also investigated the effect of the reaction time of Cas12a cleavage. At different reaction time of Cas12 cleavage, 100 fM, 10 fM, 1 fM, and 100 aM let-7a were synchronously determined by using the CAL-LAMP-based method. As shown in Figure S3, when the reaction time of Cas12a cleavage is 10 min, all samples produce weak fluorescence signals and lower concentration let-7a (100 aM and 1 fM) cannot be detected indicating low sensitivity for miRNA assay. In contrast, when the reaction time of the Cas12a cleavage is 30 min, all samples can produce strong fluorescence signals. However, the fluorescence signals produced by higher concentration let-7a cannot be distinguished well from each other. When the reaction time of the Cas12a cleavage is 20 min, all samples produce strong fluorescence signals and can be well distinguished from each other, and thus 20 min is selected as the optimizing reaction time for Cas12a cleavage.

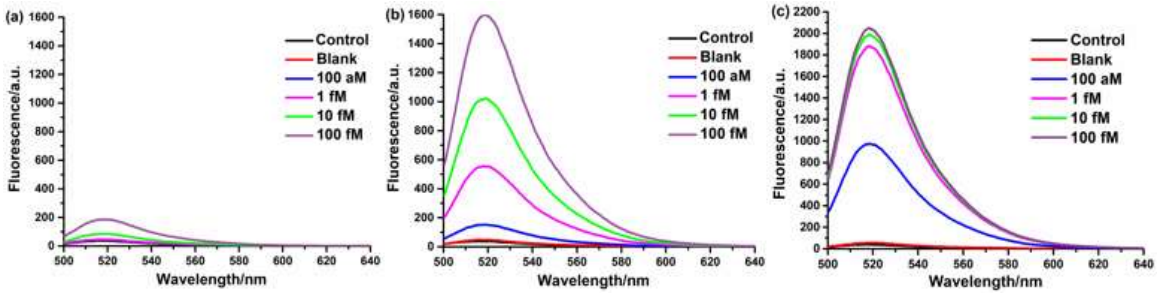

Figure S3. Effect of the reaction time of Cas12a cleavage on CAL-LAMP-based miRNA detection. The reaction time of the Cas12a cleavage is 10 min (a), 20 min (b), and 30 min (c), respectively.

5. Detection of miR-21 by CAL-LAMP.

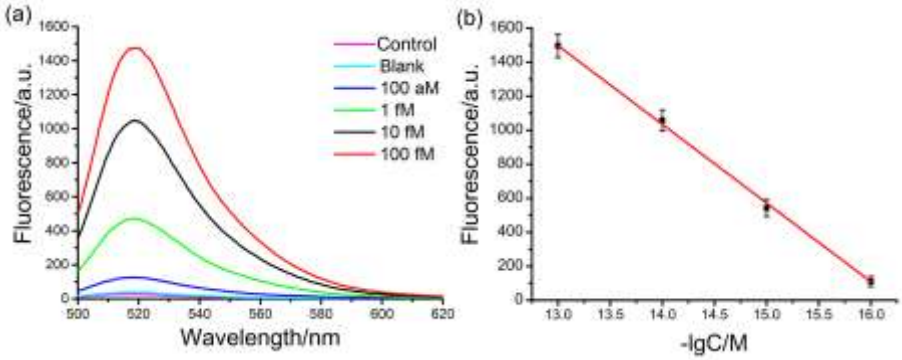

Figure S4. Detection of miR-21 in the low concentration range by CAL-LAMP. (a) Fluorescence spectra of the CAL-LAMP system in the presence of different concentrations of miR-21. miR-21 from top to bottom: 100 fM, 10 fM, 1 fM, 100 aM, 0 (blank), and control (only sgRNA/Cas12a and reporter). (b) The linear relationship between the fluorescence intensity (at 520 nm) and the concentrations of miR-21. LAMP reaction time: 23 min. The linear correlation equation $F_{520\ nm}$= 7511 + 462lg$C$(M), and the correlation coefficient R^2=0.9920. Error bars represent the standard deviation of three replicates.

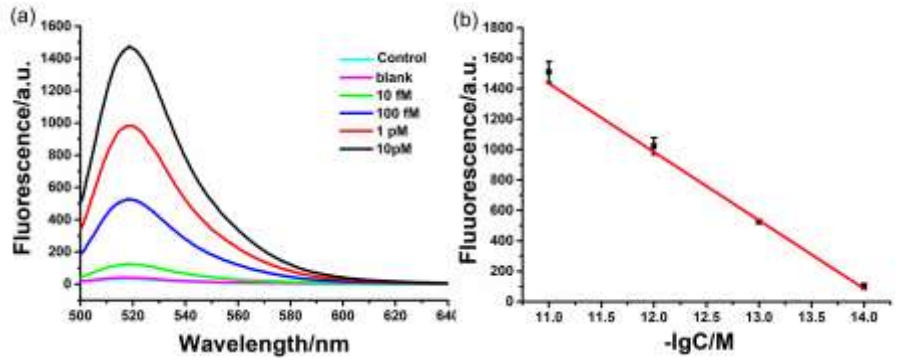

Figure S5. Detection of miR-21 in the higher concentration range by CAL-LAMP. (a) Fluorescence spectra of the CAL-LAMP system in the presence of different concentrations of miR-21. miR-21 from top to bottom: 10 pM, 1 pM, 100 fM, 10 fM, 0 (blank), and control (only sgRNA/Cas12a and reporter). (b) The linear relationship between the fluorescence intensity (at 520 nm) and the concentrations of miR-21. LAMP reaction time: 15 min. The linear correlation equation $F_{520\ nm}$= 6374 + 449lgC(M), and the correlation coefficient R^2=0.9941. Error bars represent the standard deviation of three replicates.

6. Determination results of let-7a and miR-21 in different cell extracts by CAL-LAMP and the stem-loop RT-PCR method.

Let-7a and miR-21 were also detected by the stem-loop RT-PCR-based method (*Nucleic Acids Res*. 2005, 33, e179) with the TaqMan microRNA assay kit according to the TaqMan Small RNA Assay Quick Reference (ThermoFisher Scientific).

Table S2. Determination results of let-7a and miR-21 in different cell extracts by CAL-LAMP and the stem-loop RT-PCR-based method with TaqMan microRNA assay kits.

	Amount of let-7a in 1 ng total RNA		Amount of miR-21 in 1 ng total RNA	
	CAL-LAMP	Stem-loop RT-PCR	CAL-LAMP	Stem-loop RT-PCR
MRC-5	6.8 zmol	7.5 zmol	40.7 zmol	35.8 zmol
A549	55.7 zmol	45.1 zmol	415.6 zmol	450.9 zmol
MCF-7	83.5 zmol	93.3 zmol	611.7 zmol	601.1 zmol

7. Visual detection let-7a and miR-21 in different cell extracts.

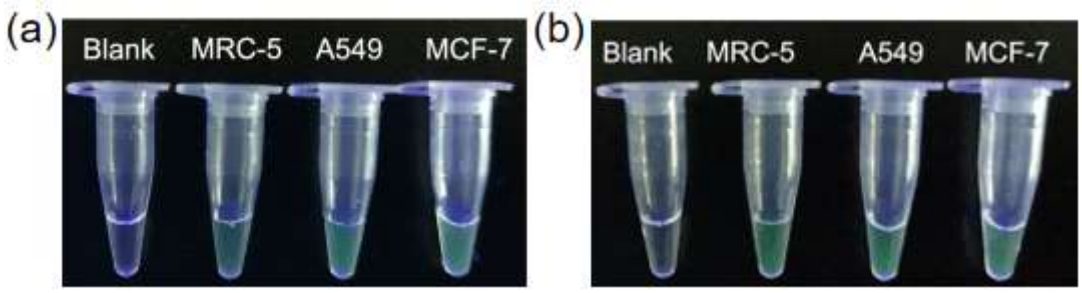

Figure S6. Visual determination of let-7a (a) and miR-21 (b) in 1 ng total RNA sample extracted from MRC-5, A549, and MCF-7 cell lines.

8. Analysis performance comparison between the proposed CAL-LAMP assay and other reported methods for the detection of miRNA.

Table S3. Analysis performance comparison between the proposed CAL-LAMP assay and other reported methods for the detection of miRNA.

Method	Required enzymes	Number of probes	Steps	Analysis Time	Detect limit	Cost per sample	Ref.
Cas-TCA	(1) SplintR ligase (2) phi29 DNA polymerase (3) T7 RNA polymerase (4) Cas12a	6	(1) HRCA, (2) Transcription (3) crRNA processing (4) Cas12-cleavage	~7 hours	1 fM	$2.7	S1
Cas12a-SCR	(1) SplintR ligase (2) T7 RNA polymerase (3) Exonuclease I (4) Cas12a	7	(1) Ligation (2) RCT (3) crRNA processing (4) Cas12-cleavage	~6 hours	100 fM	$1.9	S2
Cas12a-mediatd cascade amplification	(1) T4 RNA ligase 2 (2) T7 RNA polymerase (3) Cas12a	8	(1) Ligation (2) Transcription (3) Cleavage (4) Cas12a-cleavage	~4 hours	21.9 fM	$3.5	S3
Stem-loop RT-PCR	(1) Reverse transcriptase (2) DNA Polymerase	4	(1) Reverse transcription (2) PCR	~ 2 hours	1.3 aM	$5.7	S4
Ligation-PCR	(1)T4 RNA ligase 2 (2) Taq DNA Polymerase	4	(1) Ligation (2) PCR	~2 hours	200 aM	$1.2	S5
Cas12a enhanced RCA	(1) T4 DNA ligase (2) phi29 DNA polymerase (3) Cas12a	3	(1) Ligation (2) RCA (3) Cas12a-cleavage	~4 hours	10 fM	$3.7	S6
CAL-LAMP	(1) SplintR ligase (2) Bst 2.0 WarmStart DNA polymerase (3) Cas12a	6	(1) Ligation (2) LAMP (3) Cas12a-cleavage	~1 hour	100 aM	$1.0	This work

Note: RCT: rolling circle transcription; SCR: self-powered crRNA recruiting; RT-PCR: reverse transcription polymerase chain reaction; LAMP: loop-mediated isothermal amplification; RCA: rolling circle amplification; HRCA: hyperbranched rolling circle amplification; CAL-LAMP: CRISPR/Cas12a assisted ligation-initiated loop-mediated isothermal amplification.

References:

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