

# Rolling Circle and Loop Mediated Isothermal Amplification Strategy for Ultrasensitive miRNA Detection

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**Table S1.** Pre-ligation system.

Ingredients	Volume
target miRNA or total RNA	5 µL
Padlock probe (200 pmol/L)	1 µL
heat at 65°C for 3 min then cool down to room temperature.	
10×T4 buffer	1 µL
T4 DNA Ligase (350 U/µL)	0.2 µL
Diethyl pyrocarbonate treated water	Up to 10 µL

**Table S2.** LAMP system.

Ingredients	Volume
LAMP primer mix	1 µL
dNTP (10 mmol/L)	1 µL
10×isothermal buffer	2.5 µL
Betaine (5 mol/L)	5 µL
MgSO <sub>4</sub> (100 mmol/L)	0.5 µL
T4 ligase linked products	1 µL
ddH <sub>2</sub> O	9 µL
pre-degeneration at 95°C for 5 min, then keep on ice for another 2 min	
Bst 2.0 DNA polymerase (120 U/µL)	0.05 µL
20×Evagreen	1.25 µL
ddH <sub>2</sub> O	3.7 µL

**Table S3.** The analytical sensitivity of the RC-LAMP method (n=10).

miR-200a concentration	Numbers of positive test	RSD
1 fmol/L	10/10	7.22%
750 amol/L	10/10	4.38%
500 amol/L	10/10	14.8%
250 amol/L	4/10	NA
100 amol/L	0/10	NA

**Table S4.** Comparison of sensitivity for different miRNA assay methods.

Analytical method	Advantages/ Disadvantages	Target RNA	Detection limit
Fluorescence	Sensitivity: medium; Operation: simple; Usability: good.	Let-7a	0.08 pM <sup>[1]</sup>
		miR-126, miR-141	15 pM <sup>[2]</sup>
		miR-141	0.5 fM <sup>[3]</sup>
		miR-141	1 fM <sup>[4]</sup>
		miR-200a	500 aM <sup>[5]</sup>

Electrochemical detection	Sensitivity: excellent; Operation: complex; Usability: medium.	Let-7a	6.3 pM <sup>[12]</sup>
		miR-141	0.045 fM <sup>[7]</sup>
		miR-21	10 aM <sup>[8]</sup>
		miR-107	100 aM <sup>[9]</sup>
Surface-enhanced Raman Scattering	Sensitivity: good; Operation: complex; Usability: medium.	miR-21	1 pM <sup>[10]</sup>
		miR-133a	0.306 fM <sup>[11]</sup>
ICP-MS	Sensitivity: excellent; Operation: complex; Usability: excellent.	miR-21	41 aM <sup>[6]</sup>
UV-Vis	Sensitivity: excellent; Operation: complex; Usability: medium.	miR-21	8.6 aM <sup>[13]</sup>
Fluorescence (this work)	Sensitivity: good; Operation: simple; Usability: good.	miR-200a	500 aM

**Table S5.** The validation of the matrix effect (n=3).

Total RNA (ng)	Recovery (%)			Mean (%)	SD	RSD
	1	2	3			
250	98.4	97.2	96.3	97.3	1.05	1.08
500	96.5	96.7	99.1	97.4	1.45	1.48
1000	97.5	96.4	102	98.6	2.97	3.01
1500	82.6	85.1	76.3	81.3	4.53	5.58
2000	66.1	59.7	60.3	62.0	3.53	5.70

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