Supporting information

CRISPR-Cas12a Coupled with Cyclic Reverse Transcription for amplified detection of miRNA

Xi Long,[a] Jiacheng Li,[b] Tong Luo,[a] Hui Liu,[a] Zhiwei Deng,[a] Jiacheng Ding,[a] Zan Gong,[a] Yanjing Yang*[a] and Shian Zhong*[a]

a. College of Chemistry and Chemical Engineering, Central South University, Changsha, 410083, P. R. China.

b. State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Key Laboratory for Bio-Nanotechnology and Molecular Engineering of Hunan Province, Hunan University, Changsha, 410082, P. R. China.

*Email: yangyanjing@csu.edu.cn
zhongshian@aliyun.com

1. Materials.

The DNA and RNA used in the experiments were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). and dissolved in DEPC-treated distilled water. A first-strand cDNA synthesis kit was purchased from Thermo Fisher Scientific.

Table S1. Nucleic acid sequence used in the experiments

Oligonucleotides	Sequence（5' to 3'）
Variable primer	TAGCTTATTTACGTCGCCGTCCAGCTCGACCTAGCTTATCATCATCAGTC
Hairpin DNA	FAM-CAGTCGTTACGCTACTCGACTG-BHQ2
NTS	GGTCGAGCTGGACGGCGACG
Short DNA	CY3-TTATT-BHQ2
crRNA-1	**UAAUUUCUACUAAGUGUAGAUCGUCGCCGUCCAGCUCGACC**
crRNA-2	**UAAUUUCUACUAAGUGUAGAUUCAACAUCAUCUGAUAAGC**
miRNA-21	**UAGCUUAUCAGACUGAUGUUGA**
miRNA-122	**UGGAGUGUGACAAUGGUGUUUG**
miRNA-1a	**UGGAAUGUAAAGAAGUAUGUAU**
Single-mismatched	UAGCUUAUCAGACUGAUCUUGA
TS-R	**GGUCGAGCUGGACGGCGACG**
b5	TTTACGTCGCCGTCCAGCTCGACCTAGCTTCAACATCAG
b7	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATCAG
b8	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATTCAACATCAG
b9	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCTCAACATCAG
b10	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCATCAACATCAG
b11	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAGTCAACATCAG
a6	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACA
a8	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATC
a9	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATCA
a10	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATCAG
a11	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATCAGT
a12	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATCAGTC
a14	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATCAGTCTG
a16	TTTACGTCGCCGTCCAGCTCGACCTAGCTTATCAACATCAGTCTGAT

2. Methods

CRT reaction

A first-strand cDNA synthesis kit from Thermo Fisher Scientific was used. The reaction solution contains 0.5 μL of reverse transcriptase, 5 U of ribozyme inhibitor, 0.5 mM dNTPs, target miRNA, variable primers and buffer added according to experimental needs. 10 μL of the CRT reaction mixture was incubated in a thermostatic metal bath for 45 minutes at 42°C, followed by 5 minutes incubation at 85°C to terminate the reaction.

CRISPR-CRT reaction

10 μL of CRT reaction product was mixed with 10 μL of digestion solution. The mixed

digestion solution contains: 50 nM LbCas12a-crRNA, 1×NEBuffer 2.1, 1 μM substrate. After incubating in a thermostatic metal bath and reacting at 37°C for 1 hour, the reaction was quenched by heating at 65°C for 5 minutes.

Fluorescence assay

The solution after the reaction was diluted to 100 μL, then transferred to a fluorescence cuvette, and the fluorescence spectrum was measured on an F-2700 fluorescence spectrophotometer. The test conditions for the wavelength scan were: excitation wavelength of 485 nm, collected emission wavelengths ranging from 505 to 650 nm, wavelength interval of 1 nm, excitation slit width of 5 nm, emission slit width of 10 nm, and voltage of 70 V. The ratio of the fluorescence value at the wavelength of 518 nm in the experimental group to the fluorescence value at the wavelength of 518 nm in the blank group is the signal-to-background ratio (F/F_0).

Electrophoresis assay

The experimental results were analyzed using 20% polyacrylamide gel electrophoresis. Add 2 μL SYBR-GOLD and 2 μL loading buffer to each sample. The electrophoresis conditions were as follows: the voltage was 120 V for 30 minutes. After the electrophoresis, the images were imaged on a gel imager ImageQuant LAS 500, and the pictures were saved.

Detection in blood samples

In order to verify the detection ability in actual samples, experiments were carried out by doping the target substance into the treated serum. Serum samples from healthy individuals were obtained from Changsha Fourth People's Hospital, with the permission of the local regulatory authority and the consent of the serum sample donor. The serum was first centrifuged at 10,000×g for ten minutes at 4°C, then 50 μL of the supernatant was mixed with 50 μL of DEPC-treated distilled water, and the mixture was heated at 65°C for 10 minutes to make the mixture deoxyribozyme inactivation. Finally, different concentrations of miRNA-21 were dissolved with the treated serum from healthy donors, and the subsequent CRISPR-CRT reaction were carried out.

3. Figures

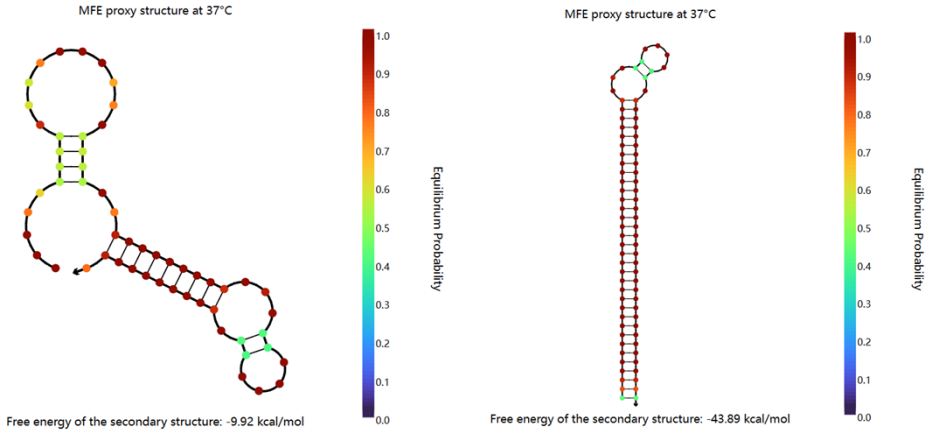

Figure S1 the predicted secondary structures of the predicted synthesized hairpin (c-b-a*-b*) and the RT products (c-b-a*-b*-c*) by NUPACK.

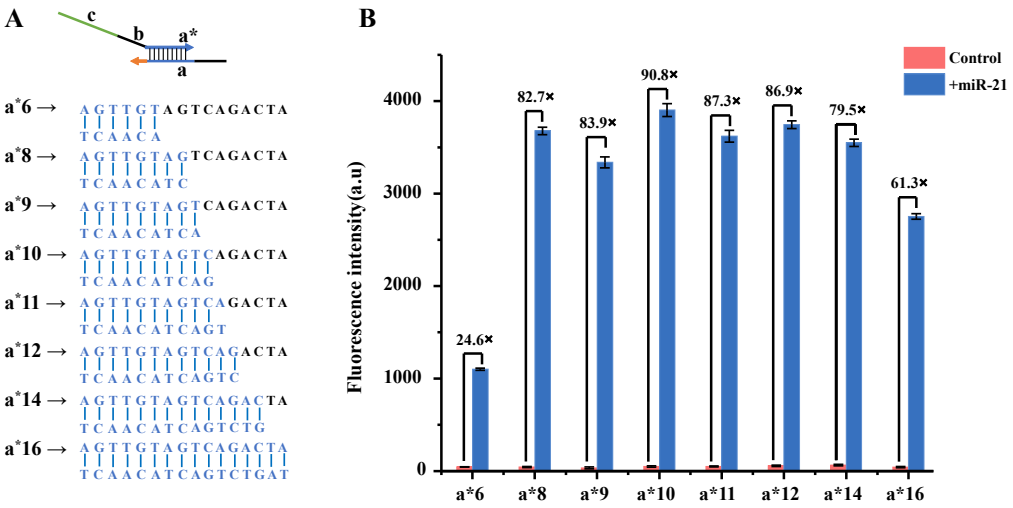

Figure S2 The variable primer with different lengths of 'a*' and the corresponding comparison of cleavage activity.

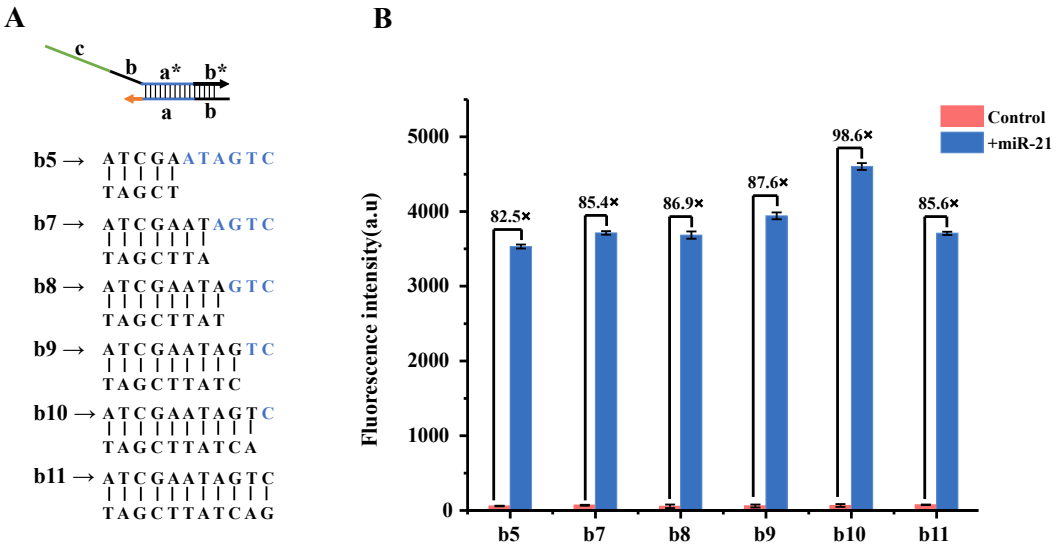

Figure S3 The variable primer with different lengths of 'b' and the corresponding comparison of cleavage activity.

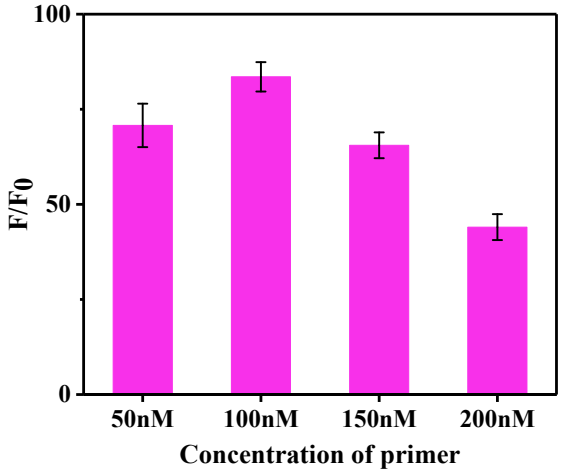

Figure S4 The effect analysis of concentration of primer to the cleavage reaction.

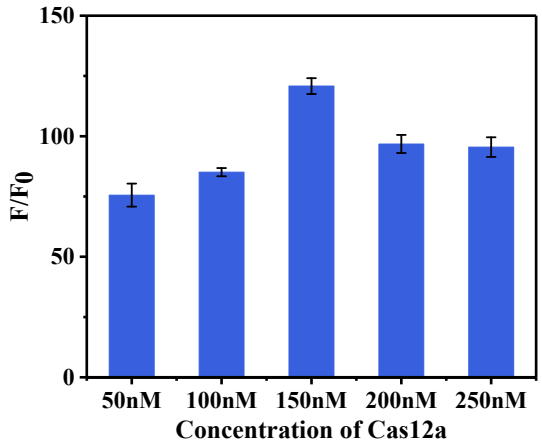

Figure S5 The effect analysis of concentration of Cas12a to the cleavage reaction.

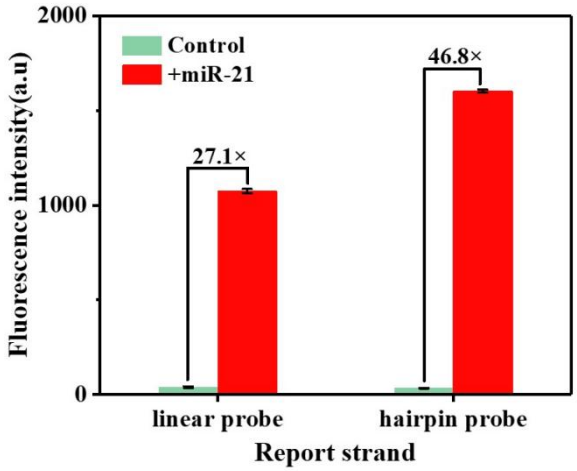

Figure S6 Comparison of fluorescence intensity between hairpin report probe and ssDNA report probe.

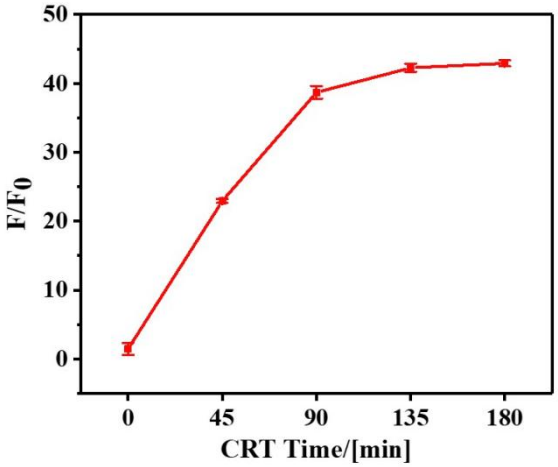

Figure S7 The effect analysis of reverse transcription time to the cleavage reaction.

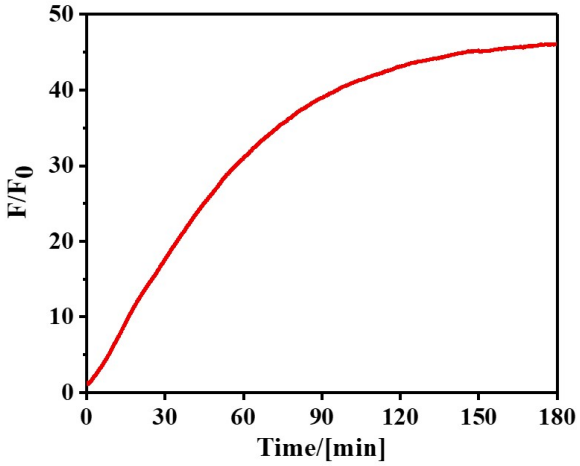

Figure S8 The effect analysis of cleavage time of Cas12a to the cleavage reaction.

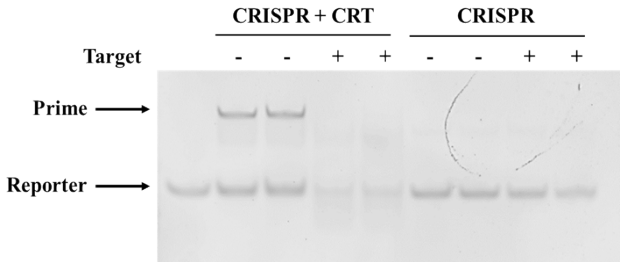

Figure S9 The electrophoresis plot to compare the cleavage efficiency of CRISPR-CRT and CRISPR.

Figure S10 (A) (B) Fluorescence assay of CRISPR-CRT and CRISPR response to different concentrations of miR-21. (C) Schematic illustration of the comparison of CRISPR-CRT with CRISPR in response to miRNA. (D) Fluorescence assay of the comparison of CRISPR-CRT with CRISPR in response to miRNA. (E) Calibration curve of CRISPR-CRT and CRISPR in response to different concentrations of miRNA-21.

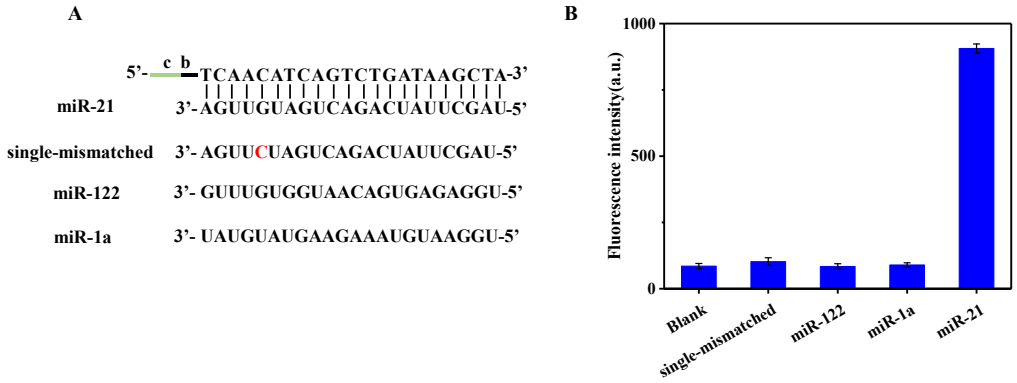

Figure S11 (A) miRNA sequences used in specificity analysis. (B) Single-base specificity analysis. The concentration of the target is 1 nM. The data are presented as mean ±s.d. of three replicate measurements.

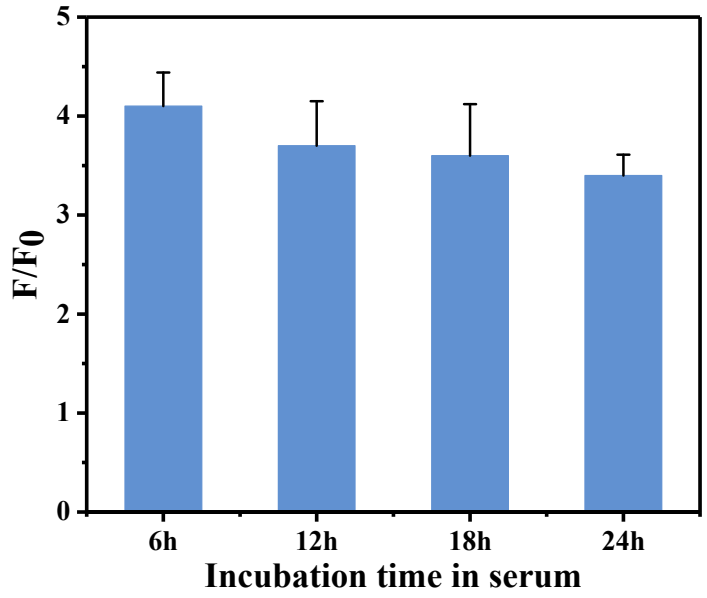

Figure S12 The F/F_0 responded to the incubation time in serum of CRISPR/Cas12a-CRT sensor under the 1nM target.

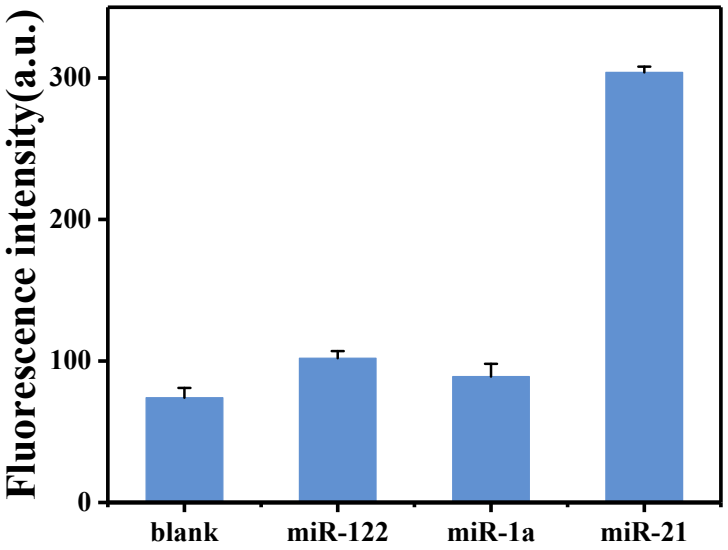

Figure S13 the specificity analysis in serum of CRISPR/Cas12a-CRT under the 1nM miR-21 in serum.

Table S1 CRISPR system-based miRNA quantity methods

Analytical technique	Target	LOD	Reference
Fluorescence	miR-299	50 fM	1
Fluorescence	Let7a	1 pM	2
Fluorescence	miRNA205	1.1 pM	3
photoelectrochemical	HPV	1.6 pM	4
Fluorescence	miR-141	82 aM	5
Fluorescence	miR-21	6.3 pM	6
Fluorescence	miR-100	0.07fM	7
photoelectrochemical	miR-21	5.8fM	8

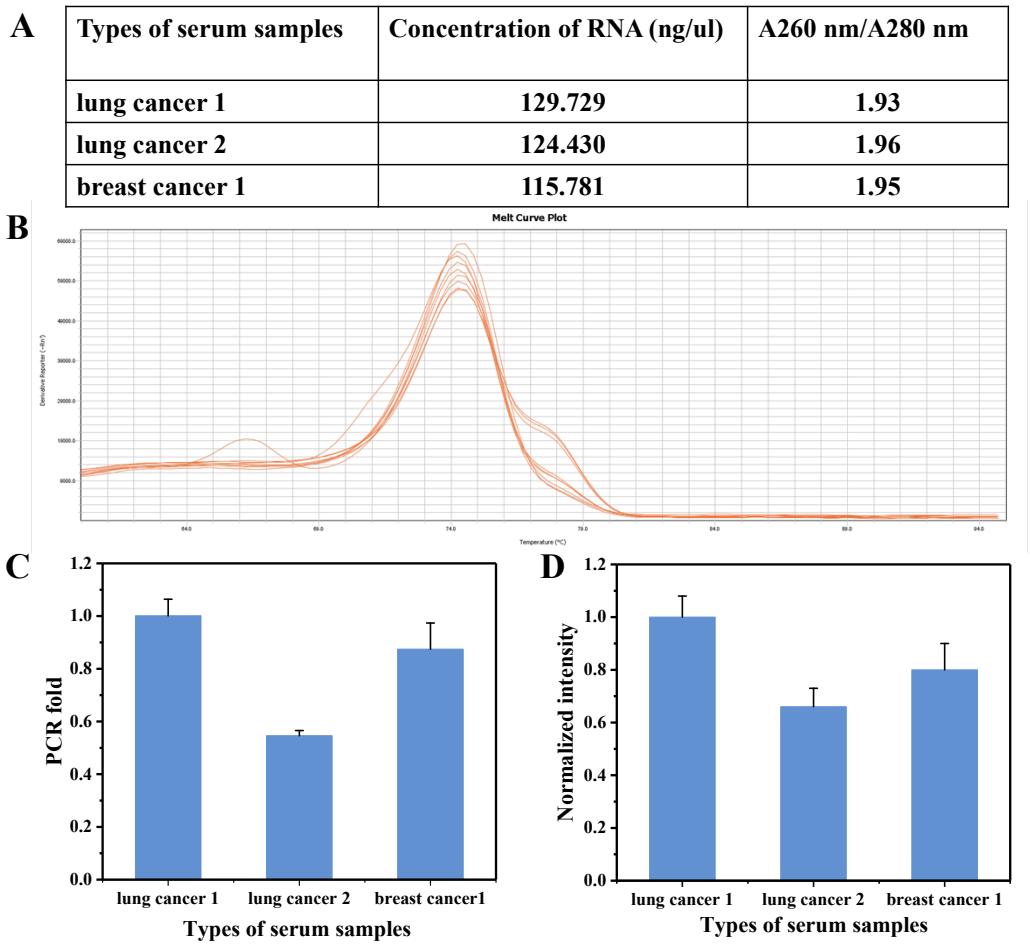

Figure S14 (A) The quantity of RNA in different serum samples. (B) Amplification plot of miR-21 by RT-qPCR（C）The relation expression level of miR-21. (D) miRNA-21 detection in disease serum samples.

Reference

1. M. Zhong, K. Chen, W. Sun, X. Li, S. Huang, Q. Meng, B. Sun, X. Huang, X. Wang, X. Ma and P. Ma, *Biosensors & Bioelectronics*, 2022, **214**, 114497.
2. Z. Guo, X. Tan, H. Yuan, L. Zhang, J. Wu, Z. Yang, K. Qu and Y. Wan, *Talanta*, 2023, **252**.
3. S. Gong, J. Li, W. Pan, N. Li and B. Tang, *Analytical Chemistry*, 2021, **93**, 10719-10726.
4. H. Gong, Y. Wu, R. Zeng, Y. Zeng, X. Liu and D. Tang, *Chemical Communications*, 2021, **57**, 8977-8980.
5. Y. Yang, J. Yang, F. Gong, P. Zuo, Z. Tan, J. Li, C. Xie, X. Ji, W. Li and Z. He, *Sensors and Actuators B-Chemical*, 2022, **367**, 132158.
6. H.-Y. Jia, H.-L. Zhao, T. Wang, P.-R. Chen, B.-C. Yin and B.-C. Ye, *Biosensors & Bioelectronics*, 2022, **211**, 114382.
7. S. Peng, Z. Tan, S. Chen, C. Lei and Z. Nie, *Chemical Science*, 2020, **11**, 7362-7368.
8. H. Shen, A. Qileng, H. Yang, H. Liang, H. Zhu, Y. Liu, H. Lei and W. Liu, *Analytical Chemistry*, 2021, **93**, 11816-11825.