

Supplemented material

Supplemented experimental section

PCR detection of miRNA-155 (Supplementary Methods)

Synthetic miRNA-155 and serum-derived miRNA samples were reverse transcribed using a stem-loop primer specific for miRNA-155 and M-MLV reverse transcriptase (Thermo Fisher) at 42 °C for 30 min. The resulting cDNA was then amplified by qPCR using a miRNA-specific forward primer, a universal reverse primer, and SYBR Green Master Mix on a real-time PCR system (Bio-Rad CFX96). The thermal cycling protocol was: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Standard curves were generated from serial dilutions of synthetic miRNA-155, and all samples were measured in triplicate.

Supplementary Table 1. Single-stranded DNA and RNA sequences used in this study

Title	Sequences (5' to 3')
Allosteric probe	TTC CCC CTA TCA CGT CCA GAA GCC CTA TAG TGA GTC GTA TTA AGG GCT TC
Assistance probe	TTA ATG CTA ATC GGT CCA GAA GCC CGC TGA GGT AGC ATT AAG AAG CCC T
crRNA	GGA CCA CCC CAA AAA UGA AGG GGA CUA AAA CUG GUC AAA CUG CAU AGG UAG UCC AGA AG
Reporter	FAM/UUU UU/BHQ1/
miRNA-155	GGG CUC UGG ACG UGA UAG GGG UU
miRNA-10b	UAC CCU GUA GAA CCG AAU UUG UG
miRNA-21	UAG CUU AUC AGA CUG AUG UUG A

Supplementary Table S2. A brief comparison of the method with former CRISPR/Cas system -based miRNA detection methods.

Methods	Mechanism	LOD	Reaction duration	Ref
The method	DNA polymerase/ endonuclease assisted signal amplification; trans-cleavage of Cas13a/crRNA	548 aM	60 min	
RACE	RCA	90 fM	120 min	1
Cas12a	RCA; trans-cleavage of Cas12a/crRNA	47 fM	100 min	2

References:

1. Wang, R.; Zhao, X.; Chen, X.; Qiu, X.; Qing, G.; Zhang, H.; Zhang, L.;

Hu, X.; He, Z.; Zhong, D.; Wang, Y.; Luo, Y., Rolling Circular Amplification (RCA)-Assisted CRISPR/Cas9 Cleavage (RACE) for Highly Specific Detection of Multiple Extracellular Vesicle MicroRNAs. *Anal. Chem.* **2020**, *92* (2), 2176-2185.

2. Zhang, G. Z., L.; Tong, J.; Zhao, X.; Ren, J., CRISPR-Cas12a enhanced rolling circle amplification method for ultrasensitive miRNA detection. *Microchem. J.* **2020**, *158* (2020), 105239.

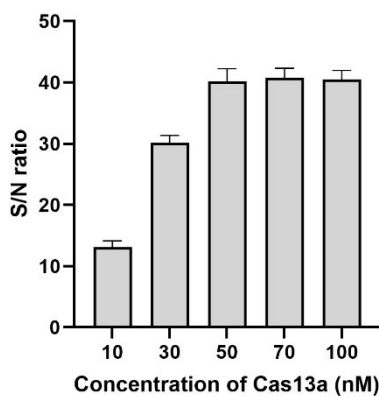


Figure S1. Signal-to-background (S/N ratio) of the method when detecting miRNA with different concentrations of Cas13a concentration.