Supplementary Material

A Bst-driven Cas12a cascade amplification strategy for microRNA detection

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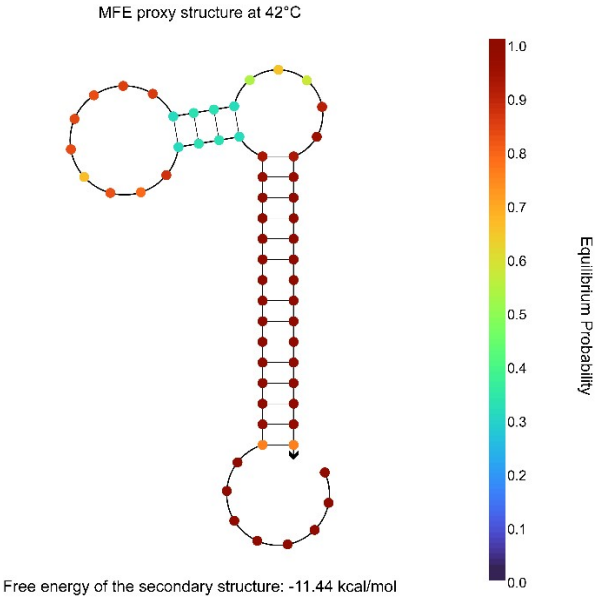

Fig. S1. Predicted secondary structure of the THP generated by NUPACK under experimental ionic conditions.

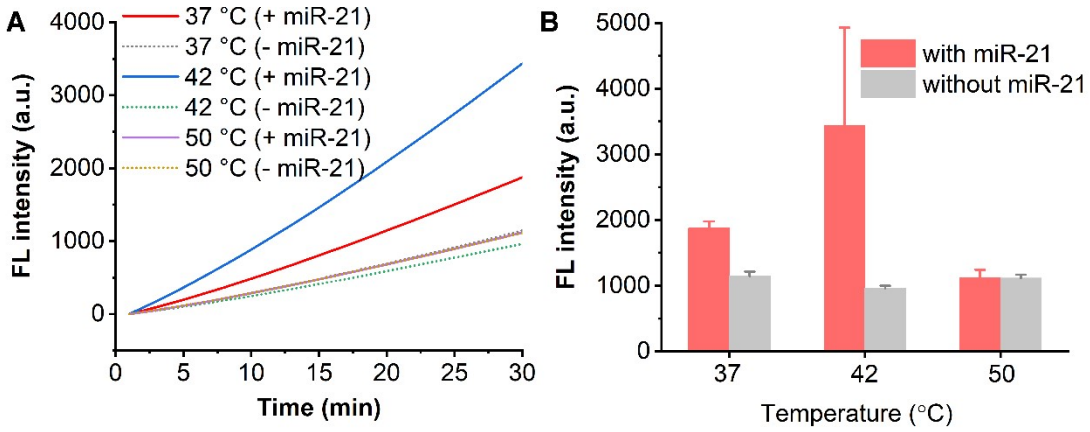

Fig. S2. (A) Real-time fluorescence curves obtained at different temperatures (37, 42, and 50 °C). (B) Corresponding fluorescence intensity comparison at the endpoint.

Fig. S3. Native PAGE analysis confirming the generation of PAM-containing dsDNA amplification products.

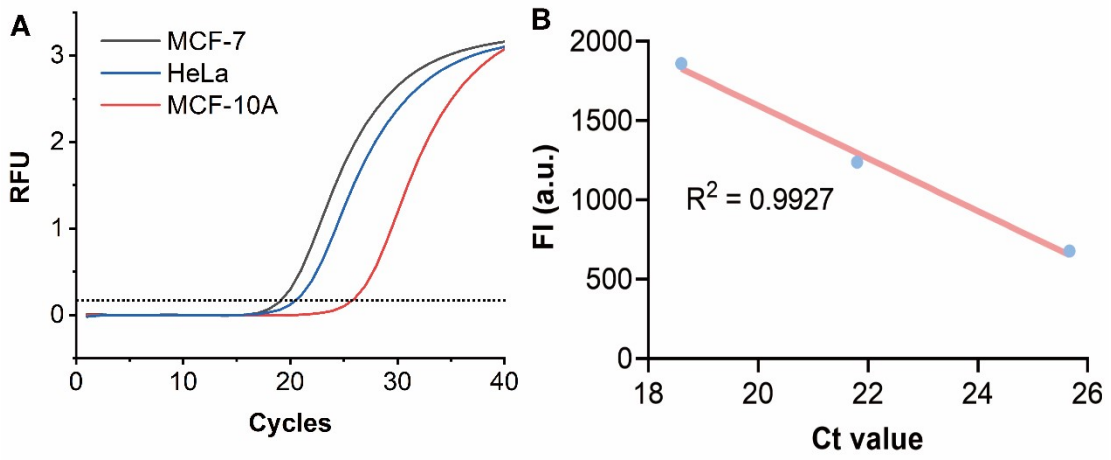

Fig. S4. (A) MiR-21 in different human cells with the RT-qPCR. (B) Correlation analysis between Bst-driven CRISPR/Cas12a fluorescence intensities and qPCR Ct values.

Table S1. Oligonucleotides utilized in the present study.

Name	Sequence (5'-3')
THP	TCAACATCAGTCTGATAAGCTATTTAGATCGTTACGCTAACTA TGATAGCTTATCAGACT-P
P	AGTCTGATAAGCTATAA
crRNA	UAAUUUCUACUAAGUGUAGAUGAUCGUUACGCUAACUAUGA
FQ-ssDNA	FAM-TTATT-BHQ1
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-101	UACAGUACUGUGAUAACUGAAG
miR-122	UGGAGUGUGACAAUGGUGUUUG
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-155	UUAAUGCUAAUCGUGAUAGGGGU

Table S2. Compositions of the buffer used in this work.

Buffer	Compositions
Buffer 1	50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/ml BSA, pH 7.9
Buffer 2	100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9
Buffer 3	50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, 1 mM DTT, pH 7.9
Buffer 4	150 mM NaCl, 20 mM Tris-HCl, 2 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 150 mM KCl, 0.1% Tween 20 pH 8.8

Table S3. Comparison of reported methods and our proposed microRNA detection strategy.

Method	linear range	Detection limit	References
Fluorescence	200 pM to 20 nM	200.00 pM	1
Fluorescence	1 nM to 16 nM	47.00 pM	2
Fluorescence	Not mentioned	10.00 pM	3
Fluorescence	10 pM-1 nM	4.8 pM	4
Fluorescence	10 pM to 1000 nM	9.25 pM	This work

Table S4. Recovery experiment for detection of miR-21.

Sample	Added (pM)	Founded (pM)[a]	RSD (%, n = 3)	Recovery (%, n = 3)
1	50.00	49.70	4.82	99.40
2	100.00	103.12	6.31	103.12
3	500.00	516.27	8.54	103.25

References

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