Supporting Information

A One-Pot CRISPR-RCA Strategy for Ultrasensitive and Specific Detection of circRNA

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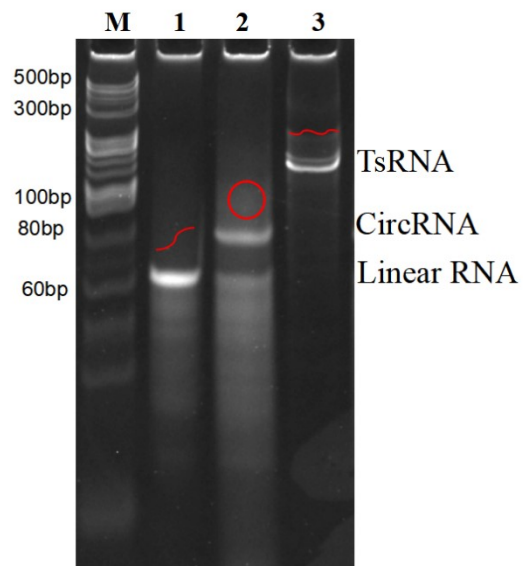
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Supplementary figures

Figure S1. (A) Schematic diagram illustrating the synthesis of circRNA1445 through the use of linear RNA with the assistance of linear RNA and T4 RNA ligase 2. (B) Native PAGE analysis of the synthesized circRNA after RNase R digestion.
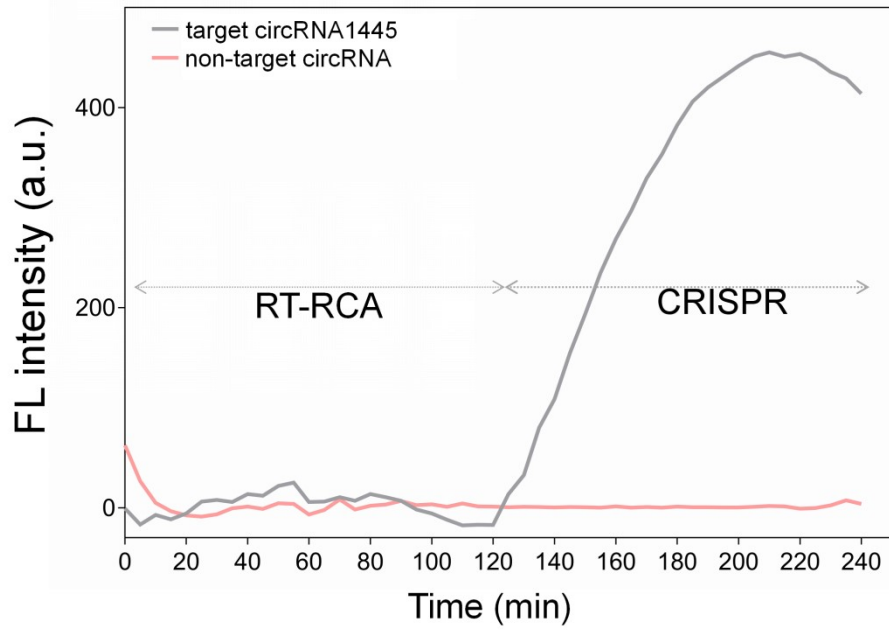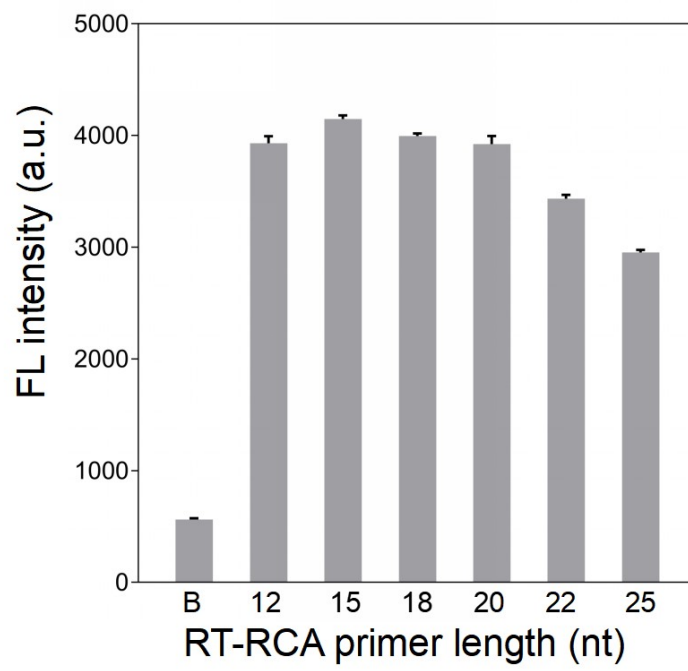
Figure S2. Denaturing PAGE was employed for the separation and purification of the obtained circRNA. Lane M: DNA marker; Lane 1: linear RNA; Lane 2: post-ligation; Lane 3: tsRNA.

Figure S3. Real-time fluorescence monitoring assay demonstrating the feasibility of the two-step assay. The gray arrow indicates the time of two-step process.

Figure S4. Fluorescence intensity as a function of primer length of the RT-RCA .
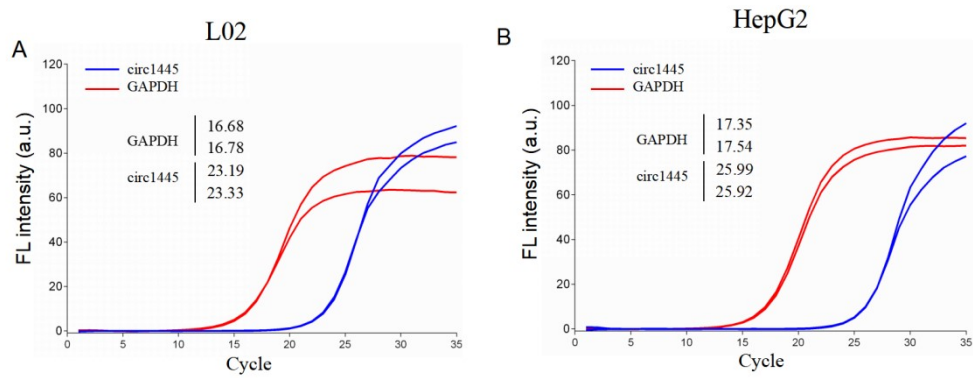
Figure S5. (A) The real-time reverse transcription PCR for circRNA1445 and GAPDH in LO2 cell. (B) The real-time reverse transcription PCR for circRNA1445 and GAPDH in HepG2 cell. GAPDH was employed as the universal endogenous control.

Supplementary tables

Table S1. DNA and RNA sequences used in this study.

Name	Sequence (5'-3')	Purpose
P-linear RNA	P-UUGGGAAAGAUGAAAUGCUUAAAACAAAAGGGAGGCUUGUGGAUCAGAAUCUGAACAAAA	For cicrRNA formation
Ligation primer	AAGCATTTCATCTTTCCCAATTTTGTTCAGATTCTGATCC	For cicrRNA formation
Linear RNA	GGAGGCUUGUGGAUCAGAAUCUGAACAAAAUUGGGAAAGAUGAAAUGCUUAAAACAAAAG	For specificity
TsRNA	GGAGGCUUGUGGAUCAGAAUCUGAACAAAAUUGGGAAAGAUGAAAUGCUUAAAACAAAAGGGAGGCUUGUGGAUCAGAAUCUGAACAAAAUUGGGAAAGAUGAAAUGCUUAAAACAAAAG	For specificity
RT primer-12nt	AAGCATTTCATC	RT-RCA
RT primer-15nt	AAGCATTTCATCTTT	RT-RCA
RT primer-18nt	AAGCATTTCATCTTTCCC	RT-RCA
RT primer-20nt	AAGCATTTCATCTTTCCCAA	RT-RCA
RT primer-22nt	AAGCATTTCATCTTTCCCAATT	RT-RCA
RT primer-25nt	AAGCATTTCATCTTTCCCAATTTTG	RT-RCA
crRNA-1	UAAUUUCUACUAAGUGUAGAU<u>AAAACAAAAGGGAGGCUUGUG</u>	Target recognition
crRNA-2	UAAUUUCUACUAAGUGUAGAU<u>GAUCAGAAUCUGAACAAAA</u>	Target recognition
crRNA-3	UAAUUUCUACUAAGUGUAGAU<u>GGAGGCUUGUGGAUCAGAA</u>	Target recognition
crRNA-4	UAAUUUCUACUAAGUGUAGAU<u>GAAAUGCUUAAAACAAAAG</u>	Target recognition
crRNA-5	UAAUUUCUACUAAGUGUAGAU<u>AAGAUGAAAUGCGGAAAACAA</u>	Target recognition
crRNA-8nt	UAAUUUCUACUAAGUGUAGAU<u>AAAGGGAG</u>	Target recognition
crRNA-10mt	UAAUUUCUACUAAGUGUAGAU<u>AAAAGGGAGG</u>	Target recognition
crRNA-16nt	UAAUUUCUACUAAGUGUAGAU<u>AAACAAAAGGGAGGCG</u>	Target recognition
crRNA-21nt	UAAUUUCUACUAAGUGUAGAU<u>AAAACAAAAGGGAGGCUUGUG</u>	Target recognition
crRNA-24nt	UAAUUUCUACUAAGUGUAGAU<u>TAAAACAAAAGGGAGGCGGGATC</u>	Target recognition
crRNA-26nt	UAAUUUCUACUAAGUGUAGAU<u>CTTAAAACAAAAGGGAGGCGGGATCA</u>	Target recognition
Target ssDNA without	AAGCATTTCATCTTTCCCAATTTTGTTCAGATTCTGAT	Cas12 reaction

PAM	CCACAAGCCTCCCTTTTGTTTT	analysis
Target ssDNA with PAM	AAGCATTTCATCTTTCCCAATTTTGTTCAGATTCTGAT CCACAAGCCTCCCTTTTGTTTTGAAACATT	Cas12 reaction analysis
Target dsDNA without PAM-F	AAAACAAAAGGGAGGCTTGTGGATCAGAATCTGAAC AAAATTGGGAAAGATGAAATGCTT	Cas12 reaction analysis
Target dsDNA with PAM-F	AATGTTTCAAAACAAAAGGGAGGCTTGTGGATCAGA ATCTGAACAAAATTGGGAAAGATGAAATGCTT	Cas12 reaction analysis
Non-target ssDNA without PAM	TGGCATTTGGCCTGGTGTGTGCAACATGTGAGCAGAT TGC	Cas12 reaction analysis
Non-target ssDNA with PAM	TGGCATTTGGCCTGGTGTGTGCAACATGTGAGCAGAT TGCGAAACATT	Cas12 reaction analysis
Non-target dsDNA with PAM-F	AATGTTTCGCAATCTGCTCACATGTTGCACACACCAG GCCAAATGCCA	Cas12 reaction analysis
C1445-1QF	CTTGTGGATCAGAATCTGAACA	QPCR for comparison
C1445-1QR	CCTCCCTTTTGTTTTAAGCA	QPCR for comparison
GAPDH-QF	AGAAGGCTGGGGCTCATTTG	For cell QPCR analysis
GAPDH-QR	GCAGGAGGCATTGCTGATGAT	For cell QPCR analysis
C1445-QF	TGGGCGAAAGTTCACTTAGAA	For cell QPCR analysis
C1445-QR	CACATGTGTTGCTCCATGTCT	For cell QPCR analysis
C1445-PF	ACTTCGAAGGAGAAGACTATAGAG	For PCR
C1445-PR	CACTTGACTCTGTATCCATTGT	For PCR

Table S2. Comparison biosensors for circRNA detection between our work and other methods.

No.	Signal readout	LOD	Time（min）	On-site diagnosis	References
1	Fluorescence	1 fM	-	No	(1)
2	Fluorescence	1.1 fM	140	Yes	(2)
3	Fluorescence	0.1 pM	180	Yes	(3)
4	Fluorescence	1 fM	30	Yes	(4)
5	Electrochemical	3.5 fM	90	No	(5)
6	Electrochemical	80 aM	10	No	(6)
7	Fluorescence	100 pM	50	Yes	(7)
8	Fluorescence	300 aM	30-120	Yes	This work

Supplementary references

References

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