A highly-efficient isothermal nano-detection platform coupling CRISPR/Cas technology for detection of circRNA

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Experimental section

Oligonucleotide sequence

Table S1 Oligonucleotide sequence

Oligonucleotide	Sequences（5'-3'）
Target	AAGCAGGAAGAAGGATGGTCCAGGCAACTCCTAGTAGTACAACGGAAGAAACAGCTACCCAGAAGGAACAGTGGTTTGGCAACAGA
Rec	TCCTATTTTCAGTGCAAGTTAGCTGTTTCTTCCGTTGTACTACTAGGTTCGTCCTGGACCATCCTTCTTCGGACGAACCTAGTATTTTTT
Primer 2	CAGCTAACTTGCACTGAAAATAGGA
Primer 1	TACTAGGTTCGT
M-Linker	CGCCGAATACGACGCCAGAGATCTACAAGAGTAGAAATTATTTTCCTATTTTCAGTGCAAGTTTT-Biotin
Template	CGCCGAATACGACGCCAGAGATCTACAAGAGTAGAAATTATTTTCCTATTTTCAGTGCAAGTTTT
Reporter	FAM-TTTTT-BHQ1
Cas12a-sg-F	TAATACGACTCACTATAGGGTAATTTCTACTCTTGTAGAT
Cas12a-sg-R	CGCCGAATACGACGCCAGAGATCTACAAGAGTAGAAATTA
Cas9n-sg-F	CCCGCGAAATTAATACGACTCACTATAGGGTTTCTTCCGTTGTACTACTGTTTTAGAGCTAGAAATAGC
Cas9n-sg-R	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
circSEPT9	TTCAGAGCCTGCCCCTGTGTCTCAGCTGCAGAGCAGGCTGGAGCCCAAGCCCCAGCCCCCTGTGGCTGAGGCTACACCCCGGAGCC
circCDYL	CTCAGGTGCCCGGCCCTGTGACTGCAGCCATGGCCACAGGCTTAGCTGTTAACGGGAAAGGTTGAAAGGATTGTTGACAAAAGGAA
miRNA-155	TTAATGCTAATCGTGATAGGGGT
miRNA-21	TAGCTTATCAGACTGATGTTGA
HOTAIR	ACAGAGCAACTCTATAATATGCTTATATTAGGTCTAGAAGAATG

Preparation of sgRNAs

Added the T7 promoter to the template of sgRNA sequence (Table S1). Then annealed and extended two ssDNAs that contained the template for sgRNA transcription. Used T7 RNA transcription kit to transcribe sgRNA at 37 °C for 4 hours, and purified the product by RNA purification kit. Finally, quantified the sgRNA and store it at -20 °C.

Klenow(3’-5’exo-) protein expression and purification

The Klenow(3'-5'exo-) expression vector pETKlenow was transformed into *E.coli* BL21 (DE3) and a single colony was selected for cultivation in LB broth supplemented with 50 μg/mL kanamycin at 37 °C for 16 hours. 1 mL of the culture was then transferred to 100 mL of LB-kanamycin broth, and the culture was incubated until the OD_{600} reached 0.4-0.6. The culture was induced with IPTG and further incubated at 16 °C for 16 hours. Bacterial pellets were collected via centrifugation at 5000 rpm. Following washing, the pellets were resuspended in Buffer I (50 mM Tris-HCl, pH 7.5, 300 mM NaCl). The organisms were lysed using an ultrasonic cell crusher, and the supernatant was obtained by centrifugation. An appropriate volume of nickel affinity chromatography medium was taken and loaded onto a column. The column was washed twice with Buffer I. After completing the washing steps, the supernatant was transferred to the column. The column was eluted with buffer II (50 mm Tris-HCl, pH 7.5, 300 mM NaCl , 20-300 mM imidazole) at concentrations of 20 mM, 35 mM, 50 mM, 100 mM, 200 mM and 300 mM, each for 20 column volumes. This was followed by SDS-PAGE analysis. Each gradient dialysis was performed for two hours to remove imidazole from the solution gradually. Eventually, the Klenow(3'-5'exo-) protein was stored in storage buffer containing 25 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM DTT and 25% glycerol. The concentration of Klenow(3'-5'exo-) was subsequently determined to be 10.97 μM using the BCA method.

PAGE gel electrophoresis

To preliminarily confirm the successful construction of the detection platform, the sample was mixed with 5×Loading Buffer and then spotted onto a 10% PAGE gel. Then, the gel was run in 1× TBE buffer at a constant voltage of 100 V for 45 minutes. Subsequently, the gel was imaged using a gel documentation system.

Optimisation of Klenow(3’-5’exo-) reaction conditions

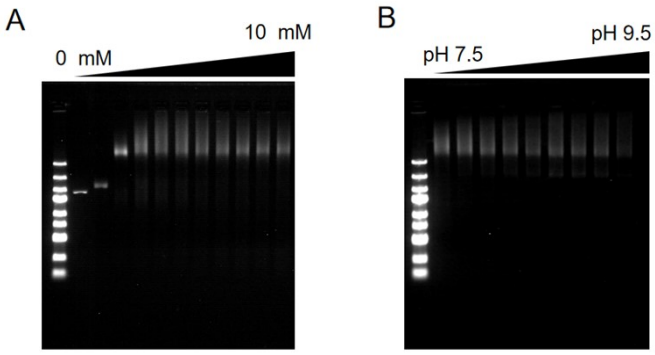

Fig S1 Effect of Mg^{2+} and pH on Klenow(3’-5’exo-) enzyme activity

(A) Effect of Mg^{2+} on Klenow(3’-5’exo-) enzyme activity and Mg^{2+} concentrations were 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mM, respectively. (B) Effect of pH on Klenow(3’-5’exo-) enzyme activity and pH was 7.5, 7.75, 8.0, 8.25, 8.5, 8.75, 9.0 , 9.25 and 9.50, respectively.

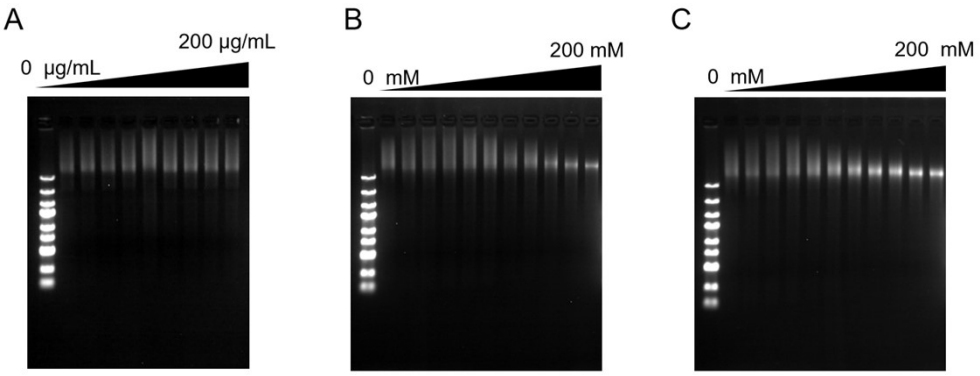

Fig S2 Effect of BSA, Arg and Lys on Klenow(3'-5'exo-) enzyme activity

(A) Effect of BSA on Klenow(3'-5'exo-) enzyme activity and BSA concentrations were 0, 25, 50, 75, 100, 125, 150, 75 and 200 μg/mL, respectively. (B-C) Effect of Arg and Lys on Klenow(3'-5'exo-) enzyme activity and concentrations were 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mM, respectively.

Construction of detection method

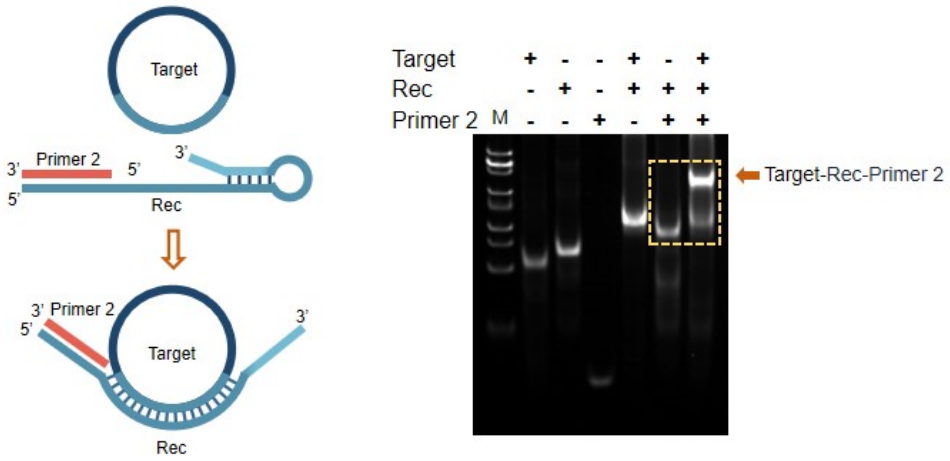

Fig S3 Construction and functional verification of Rec

Optimization of conditions for the fluorescence detection method

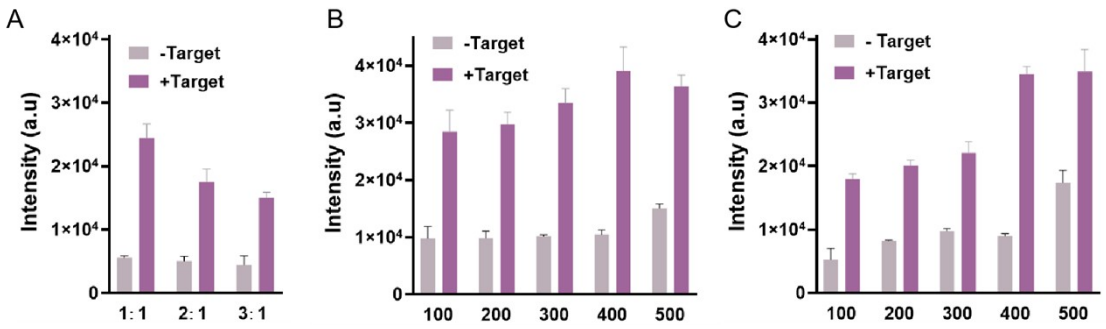

Fig S4 Optimization of oligonucleotide strand ratio and concentration

(A) Optimization of ratio between Rec and Primer 2 in the detection system. (B) Optimization of Primer 1 concentration in the detection system. (C) Optimization of Rec-Primer 2 concentration in the detection system.

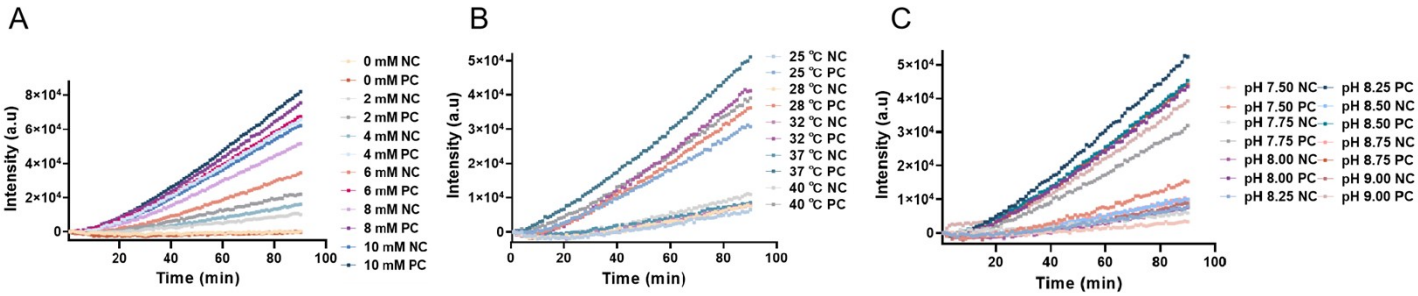

Fig S5 Effect of Mg^{2+}, temperature and pH on the detection system

(A) Effect of Mg^{2+} on the detection system. (B) Effect of temperature on the detection system. (C) Effect of temperature on the detection system. The PC experimental group refers to the system with Target and the NC experimental group refers to the system without Target.