Electronic Supporting Information

Real-time Quantification of Fusion Transcripts with Ligase Chain Reaction by Direct Ligation of Adjacent DNA Probes at Fusion Junction

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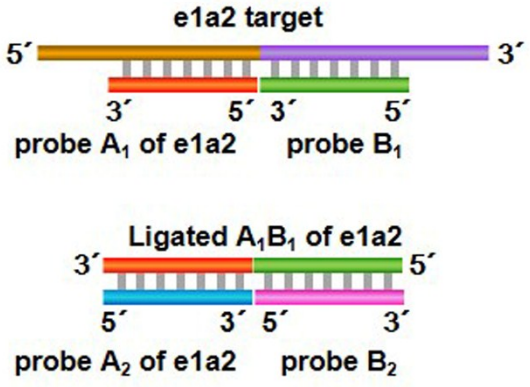
Table S1. Sequences of the RNA targets and DNA probes used in the experiments

Name	Sequence (5′-3′)
e1a2 target	GAGGGCGCCUUCCAUGGAGACGCAGAAGCCCUU CAGCGGCCAGUAGCAUCU
probe A_1 of e1a2	PO_4-CTGCGTCTCCATGGAAGGCGCC
probe A_2 of e1a2	GGCGCCTTCCATGGAGACGCAGA
e13a2 target	AGCAUUCCGCUGACCAUCAAUAAGGAAGAAGCC CUUCAGCGGCCAGUAGCA
probe A_1 of e13a2	PO_4-CTTCCTTATTGATGGTCAGCGGAATGCTGT
probe A_2 of e13a2	ACAGCATTCCGCTGACCATCAATAAGGAAGA
e14a2 target	CCACUGGAUUUAAGCAGAGUUCAAAAGCCCUUC AGCGGCCAGUAGCAUCUG
probe A_1 of e14a2	PO_4-TTGAACTCTGCTTAAATCCAGTGGCTGAGTG
probe A_2 of e14a2	CCACTCAGCCACTGGATTTAAGCAGAGTTCAAA
e19a2 target	CACUGAAGGCAGCCUUCGACGUCAAAGCCCUUCA GCGGCCAGUAGCAUCUG
probe A_1 of e19a2	PO_4-TGACGTCGAAGGCTGCCTTCAGTGC
probe A_2 of e19a2	GCACTGAAGGCAGCCTTCGACGTCAA
Probe B_1 of all targets	ATGCTACTGGCCGCTGAAGGGCTT
probe B_2 of all targets	PO_4-AGCCCTTCAGCGGCCAGTAGCAT

Note: The four fusion transcripts (e1a2, e13a2, e14a2 and e19a2) contain partial the same sequence derived from ABL gene (Scheme 1B), so the DNA probe B_1 and probe B_2 for the four targets are universal.



Scheme. S1 The orientation and complementarity of the DNA probes for detection of e1a2 target.

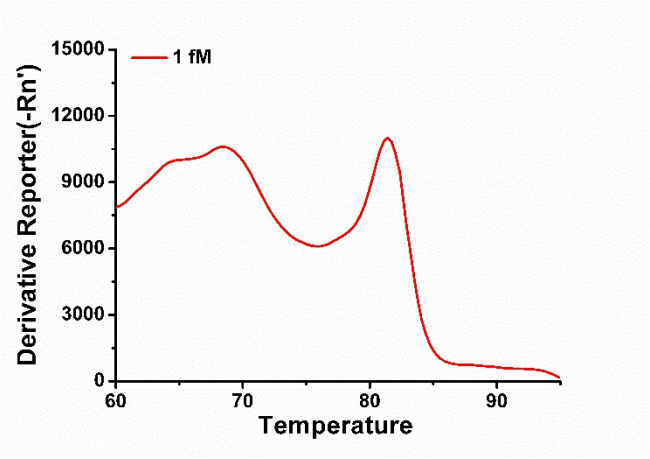


Fig S1 Melting curve profile of the LCR products by using 1 fM e1a2 as template.

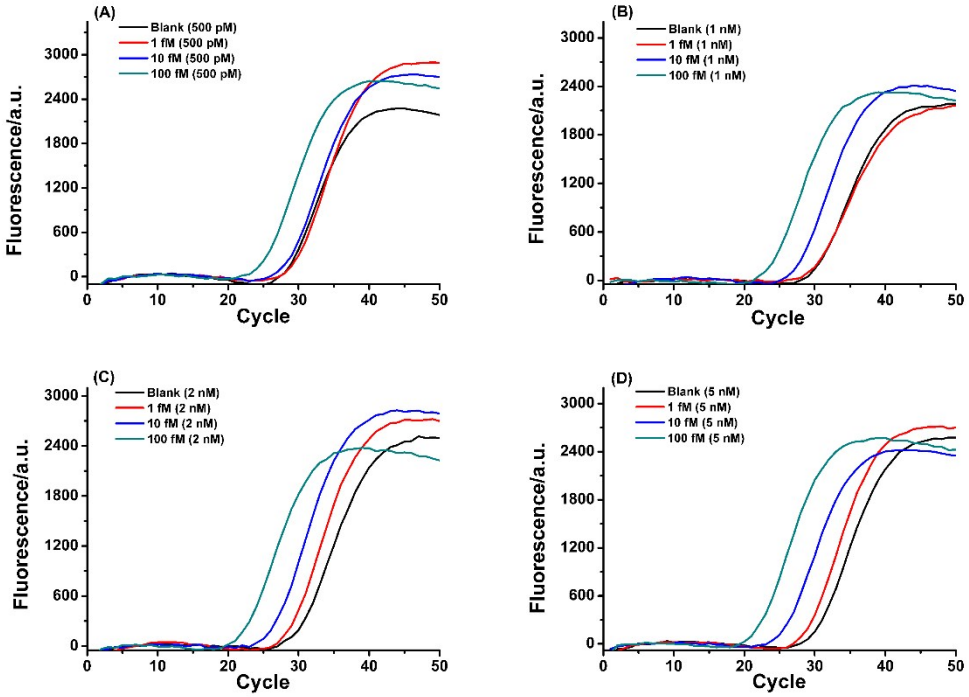


Fig. S2 Influence of the concentration of the DNA probes in the direct ligation step. The real-time fluorescence curves were produced from 0 (Blank), 1 fM, 10 fM, 100 fM of e1a2 with 500 pM (A), 1 nM (B), 2 nM (C) and 5 nM (D) adjacent probes (A_1 and B_1) in the direct ligation reaction, respectively.

The ligated product in the direct ligation is the template of the following LCR amplification, so the concentration of ligation probes is a curial factor to optimize. 500 pM, 1 nM, 2 nM and 5nM DNA probe A_1 and B_1 in the direct ligation reaction were ligated in presence of the blank, 1 fM, 10 fM and 100 fM of e1a2, respectively. As shown in Fig. S2, when the concentration of DNA probes is less than 2 nM, 1 fM of e1a2 cannot be distinguished from blank, indicating 500 pM and 1 nM of probes were insufficient. When the concentration of probes increased to 2 nM, 1 fM can be well detected by the proposed method. Meanwhile, 5 nM of probes made no significant difference with 2 nM of probes. Based on the principle of economy, the concentration of the direct ligation probes was selected as 2 nM at last.

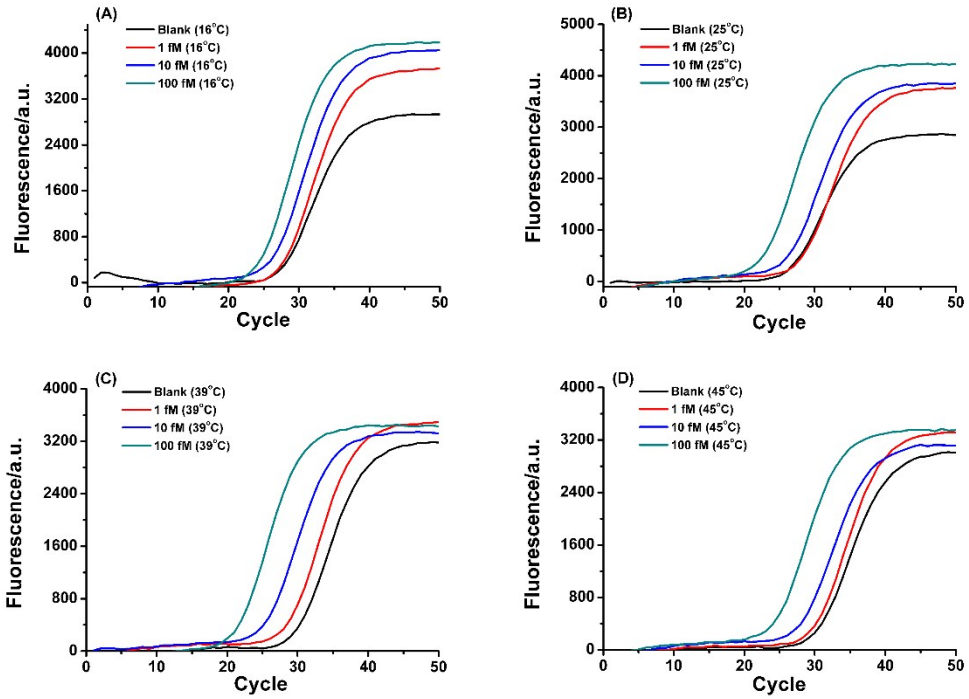


Fig. S3 Effect of ligation temperature in the direct ligation step. The real-time fluorescence curves were generated from the e1a2 with direct ligation at 16℃(A), 25℃(B), 39℃ (C) and 45℃(D), respectively. The concentration of e1a2 was Blank, 1 fM 10 fM and 100 fM, respectively.

Temperature is a key factor, which not only influences the efficiency of the probe hybridization, but also affects the function of the enzyme. Accordingly, the ligation temperature was optimized at 16℃, 25℃, 39℃ and 45℃, respectively. As shown in Fig S3, the POI value of 100 fM at 25℃ was smaller than that at 16℃, due to SplintR ligase is more active at 25℃. When the temperature increased to 39℃, the POI value of 100 fM unchanged, while the POI value of blank increased, indicating 39℃ reduced non-specific ligation. However, 45℃ is too high to the catalytic ability of SplintR ligase. As a result, 39℃ was the optimal ligation temperature for the proposed detection.

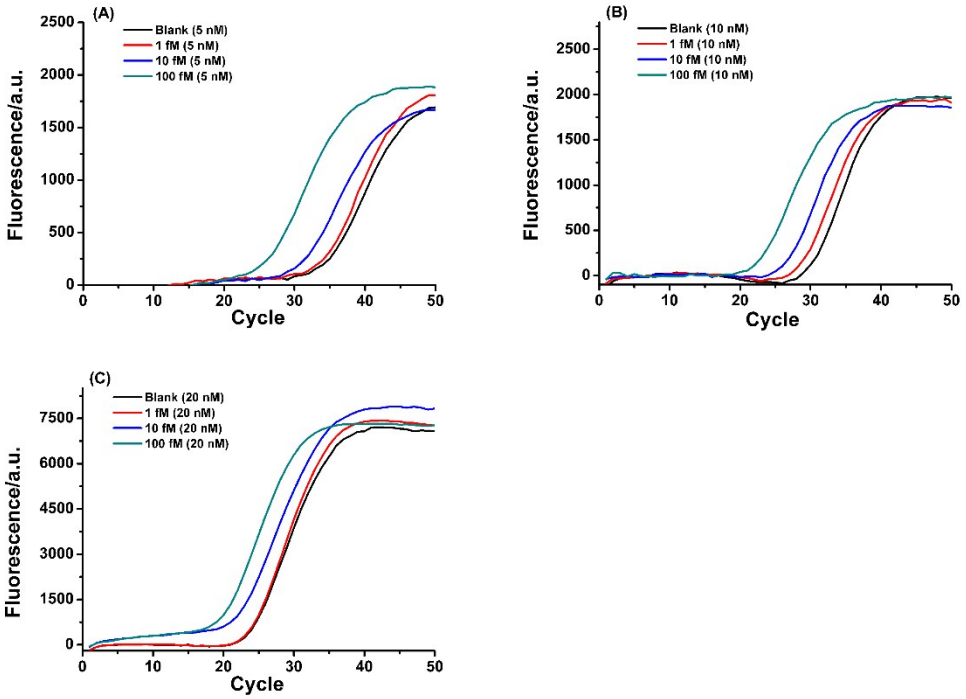


Fig. S4 Influence of the concentration of DNA probes in LCR amplification. The LCR-based method and the fluorescence measurements were carried out with (A) 5 nM, (B) 10 nM and (C) 20 nM of DNA probes in LCR reaction. The concentration of e1a2 mRNA was Blank, 1 fM 10 fM and 100 fM,, respectively.

The concentration of the four DNA probes in LCR was a crucial factor for the proposed assay. Therefore, the optimal concentration of DNA probes was investigated with 5 nM, 10 nM and 20 nM. As shown in Fig. S4, when 5 nM of probes were used, 1 fM of e1a2 produced the same POI value with blank, indicating the concentration of probes was too low to discriminate 1 fM of e1a2 with blank. 10 nM of probes gave a better discrimination between 1 fM of e1a2 and blank. With further increasing the probe concentration, the POI value of blank reduced and 1 fM of e1a2 mRNA was undetectable, indicating the non-specific ligation increased. Finally, 10 nM was selected as the optimal concentration of probes for the LCR-based method.

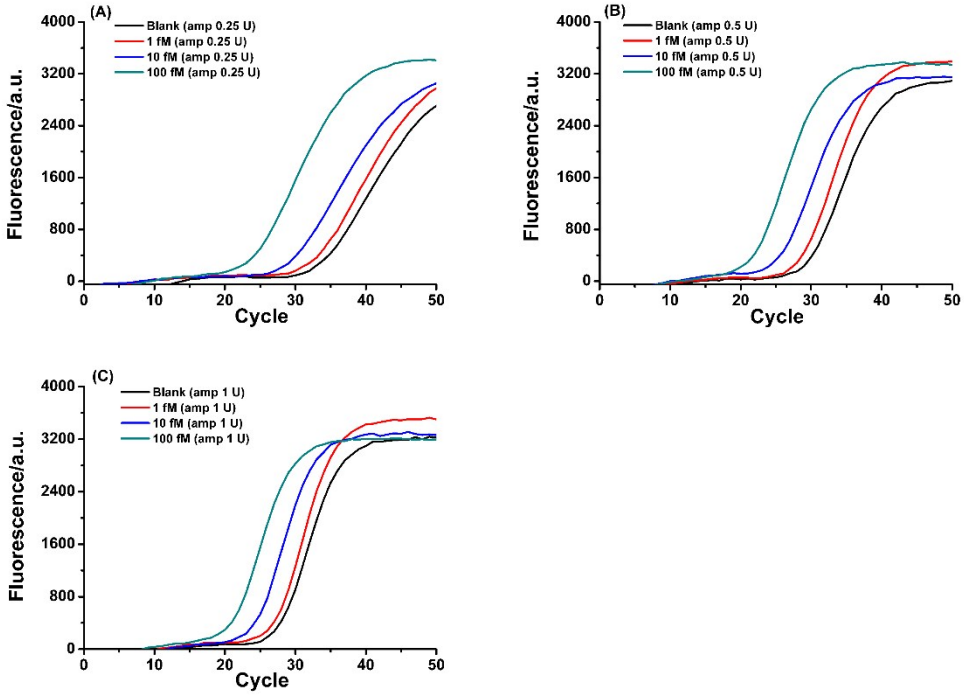


Fig. S5 Influence of the amount of Ampligase in the proposed assay. The real-time fluorescence curves were produced from e1a2 by catalyzed with 0.25 U (A), 0.5 U (B) and 1 U (C) Ampligase. The concentration of e1a2 was Blank, 1 fM 10 fM and 100 fM, respectively.

Ampligase is the catalyst in the LCR amplification, the amount of which is very important. The LCR-based detection was carried out under the catalysis of 0.25 U, 0.5 U and 1 U of Ampligase. The insufficient amount (0.25 U) Ampligase was used, so the slope of real-time fluorescent curves in Fig. S5A was obvious smaller than that in Fig. S5B and S5C. Well-define fluorescence curves were obtained by increasing the amount of Ampligase to 0.5 U. When further increasing the amount of Ampligase to 1 U, the POI value of the fluorescence curve of blank reduced, implying nonspecific reaction improved. Accordingly, 0.5 U was chosen as the optimal dosage for the LCR-based method.