Electronic Supplementary Information

One-step and highly sensitive quantification of fusion gene with isothermal amplification initiated by fusion-site anchored stem-loop primer

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1. Table S1. The sequences of the oligonucleotides used in this work

ID	Sequences (5′-3′ direction)
e13a2 fragment	UGUGUGAAACUCCAGACUGUCCACAGCAUUCCGCUGACCAU CAAUAAGGAAG*AAGCCCUUCAGCGGCCAGUAGCAUCUGA
e13a2-SLP1	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGATTTT CCTCTGCTGTCGTTTTCCGCTGAAGGGCTTCTTCCT
e13-SLP2	ATCGTCGTGACTGTTTGTAATAGGACAGAGCCCCGCACTTCA GTCACGACGATTTTAGCATTCCGCTGACCATCAATA
e13-BP	TGAAACTCCAGACTGTCCA
FIP	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGA
BIP	ATCGTCGTGACTGTTTGTAATAGGACAGAGCCCCGCAC
e1a2 fragment	ACCGGGCAGATCTGGCCCAACGATGGCGAGGGCGCCUUCCA UGGAGACGCAG*AAGCCCUUCAGCGGCCAGUAGCAUCUGA
e14a2 fragment	GGGTTTCTGAATGTCATCGTCCACTCAGCCACUGGAUUUAAG CAGAGUUCAA*AAGCCCUUCAGCGGCCAGUAGCAUCUGA
e14a2-SLP1	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGATTTT CCTCTGCTGTCGTTTTGCCGCTGAAGGGCTTTTGAAC
e14-SLP2	ATCGTCGTGACTGTTTGTAATAGGACAGAGCCCCGCACTTCA GTCACGACGATTTTCAGCCACTGGATTTAAGCAGA
e14-BP	TTCTGAATGTCATCGTCCA
e19a2 fragment	GTGTCCGGTGTGGCCACGGACATCCAGGCACUGAAGGCAGC CUUCGACGUCA*AAGCCCUUCAGCGGCCAGUAGCAUCUGA
RT-Primer ABL	TCAGATGCTACTGGCCGCTG
e13 Primer	GTGAAACTCCAGACTGTCCAC
e14 Primer	GTCATCGTCCACTCAGCCAC
e13a2-TaqMan probe	FAM-AAGGGCTTCTTCCTTATTGA-MGBNFQ
e14a2-TaqMan probe	FAM-AAGGGCTTTTGAACTCTGCT MGBNFQ

The symbol '*' indicates the fusion junction site.

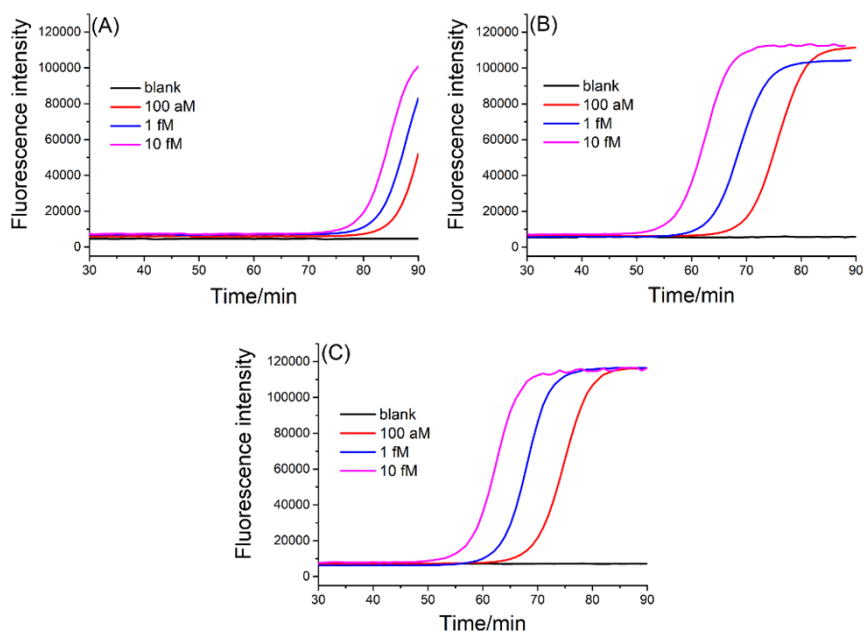## 2. Optimization of the concentration of stem-loop primers

The proposed method for fusion gene assay is based on stem-loop primer-mediated isothermal exponential amplification. Thus, the concentration of stem-loop primers will affect the reaction performance. To investigate the influence of the dosage of stem-loop primers, 20 nM, 2 nM, and 200 pM e13a2-SLP1 and e13-SLP2 were investigated by detecting the blank, 10 fM, 1 fM and 100 aM e13a2 with the proposed assay. As depicted in Fig. S1 (A-C), as the concentration of e13a2-SLP1 and e13-SLP2 increased from 200 pM to 20 nM, the POI values of the e13a2 gradually decreased *vs* no effect on the blank. The amount of stem-loop primers affects their hybridization efficiency with the target matching sequences and consequently affects the efficiency of reverse transcription and nucleic amplification. So, increasing the primer amounts can result in more double stem-loop DNA and a high speed of LAMP leading to decreased POI values. One can see from Fig. S1, the differences of the POI values between 10 fM, 1 fM and 100 aM e13a2 using 2 nM SLP1 and SLP2 were greater than that using 20 nM. Therefore, taking into consideration of detection sensitivity and detection time, 2 nM stem-loop primers were selected for the poposed assay.
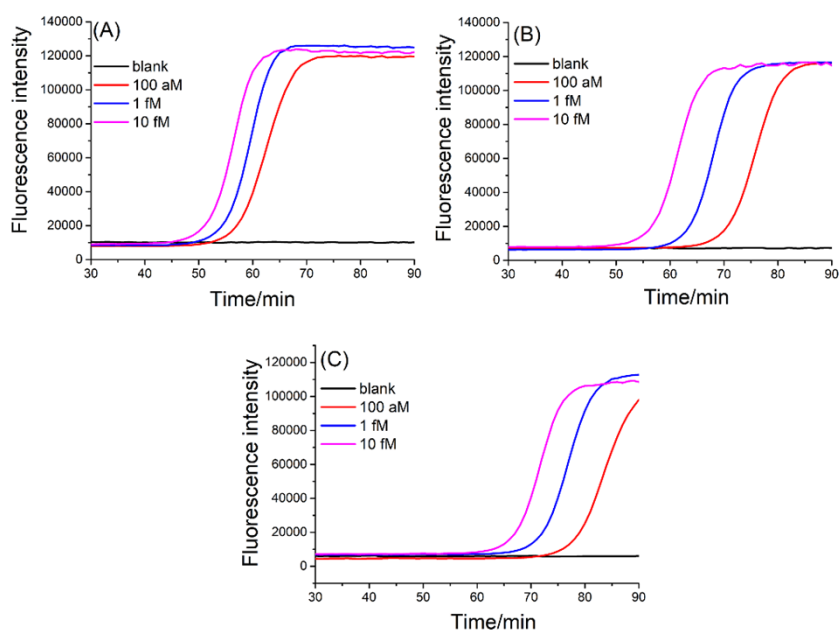
Fig. S1 The effect of the dosage of stem-loop primers on the fusion genes assay. The dosage of stem-loop primer is 20 nM (A), 2 nM (B) and 200 pM (C).

## 3. Optimization of the concentration of reverse transcriptase

Reverse transcriptase dosage can affect the efficiency of the transcript reaction. Therefore, the dosage of WarmStart RTx reverse transcriptase was optimized by detecting blank and e13a2 at 100 aM, 1 fM, and 10 fM, respectively with the proposed assay. 7.5 U, 15 U, and 20 U WarmStart RTx reverse transcriptase were investigated. When the amount of reverse transcriptase is 7.5 U, it can be seen from Fig. S2A that the POI values show a smaller difference between the different concentrations of e13a2 compared with that shown in Fig. S2B. In addition, the reaction rate is also slowed down. This is because the low amount of reverse transcriptase leads to a decrease of the reverse transcript efficiency. When the amount of reverse transcriptase is 15 U or 20 U, the reverse transcript efficiency increases and the difference in POI value between the different concentrations of e13a2 reached the maximum. These results suggest that 15 U of reverse transcriptase is sufficient for fusion gene analysis. Taking into consideration of detection sensitivity and the cost of reagent, 15 U was selected as the optimum amount of reverse transcriptase for the fusion gene assay.

Fig. S2 The effect of the dosage of reverse transcriptase on the fusion genes assay. The dosage of reverse transcriptase is 7.5 U (A), 15 U (B) and 20 U (C).

4. Optimization of the concentration of Bst DNA polymerase

The amplification reaction depends on the synthesis of auto-cycling strand displacement DNA by Bst DNA polymerase with displacement activity. Therefore, the concentration of Bst DNA polymerase is an important parameter for the fusion gene assay. The effect of the amount of Bst DNA polymerase was investigated by detecting blank and e13a2 at 100 aM, 1 fM, and 10 fM, respectively. As shown in Fig. S3 (A-C), with increasing the amount of Bst DNA polymerase from 2 ~ 8 U, the amplification reaction was correspondingly accelerated, resulting in the decrease of POI values of real-time fluorescence curves. However, the difference in the POI values between 100 aM, 1 fM and 10 fM e13a2 gradually decreased when Bst DNA polymerase was increased from 4 U to 8 U. When 2 U polymerase was used, the amplification reaction required > 90 min to generate a detectable signal. Taking into consideration of both reaction time and detection sensitivity, 8 U was selected as the optimum amount of Bst DNA polymerase for the fusion gene assay.
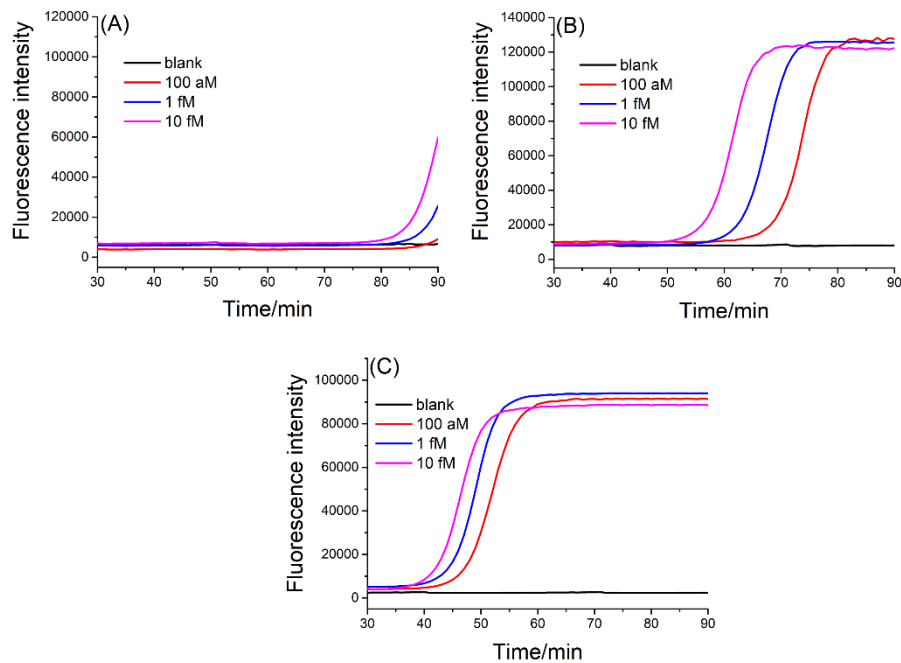
Fig. S3 The effect of the dosage of Bst DNA polymerase on the fusion genes assay. The dosage of Bst DNA polymerase is 2 U (A), 4 U (B) and 8 U (C).

5. The reaction performance of e13a2 and e14a2 assay by RT-PCR.
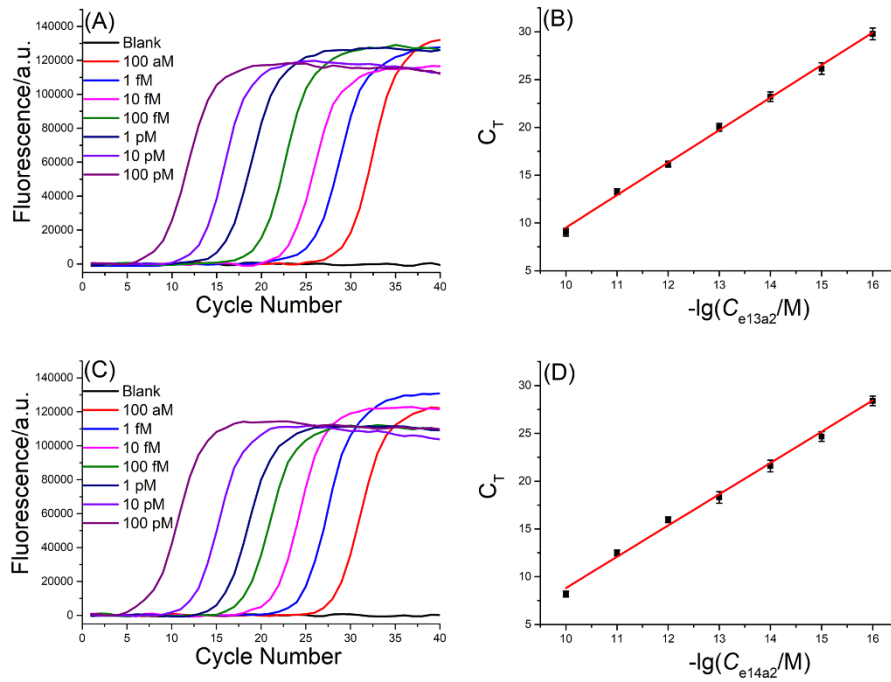
Fig. S4 Analysis performance of e13a2 and e14a2 assay by RT-PCR. (A) and (C): Real-time fluorescence amplification curves generated by different concentrations of synthetic e13a2 and e14a2 fusion transcript fragments of BCR-ABL, respectively. The amount of e13a2 from left to right was 100 pM, 10 pM, 1 pM, 100 fM, 10 fM, 1 fM 100 aM, and blank. The blank has the same detection steps as e13a2. (B) and (D): The linear relationship between the C_T values and the negative logarithm (-lg) of e13a2 and e14a2 concentrations, respectively. The fitted equations are $C_T=-24.50-3.40\lg C_{e13a2}$ (M) with a correlation coefficient of $R^2=0.9965$ and $C_T=-23.90-3.27\lg C_{e14a2}$ (M) with a correlation coefficient of $R^2=0.9924$. Each test was repeated three times. Error bars represent standard deviations of parallel assays.

6. Table S2. Comparison of the proposed strategy with previously reported methods for fusion gene detection

Target	Operating steps	Detection limits	Detection mode	Reference
EML4-ALK variant	Multi-step	4.9×10^{-5} ng/μL	Fluorescence	*Chem. Commun.* **2022**, *58*, 7618-7621.
BCR-ABL fusion gene	Multi-step	100 aM	Visual detection	*Anal. Chem.* **2019**, *91*, 12428-12434.
PML-RARα fusion gene	Multi-step	100 aM	Real-time fluorescence	*Analyst.* **2022**, *147*, 2207-2214.
BCR-ABL fusion gene	Multi-step	10 fM	Fluorescence	*Analyst.* **2018**, *143*, 4974-4980.
BCR-ABL fusion gene	Multi-step	0.27 fM	ECL	*Biosens. Bioelectron.* **2022**, *210*, 114287
BCR-ABL fusion gene	One	100 aM	Real-time fluorescence	*This work*