Supporting Information

Visual Detection of Fusion Genes by Ligation-Triggered Isothermal Exponential Amplification: A Point-of-Care Testing Method for Highly Specific and Sensitive Quantitation of Fusion Genes with a Smartphone

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Content list

1. Table S1

Table S1. The sequences of the RNAs, the DNA probes, and primers were used in this study.

name	sequence (5′-3′)
e1a2 fragment	rArGrGrGrCrGrCrCrUrUrCrCrArUrGrGrArGrArCrGrCrArG*rArArGrCrCrC
	rUrUrCrArGrCrGrGrCrCrArGrUrArGrCrA
e13a2 fragment	rArUrCrCrGrCrUrGrArCrCrArUrCrArArUrArArGrGrArArG*rArArGrCrCrC
	rUrUrCrArGrCrGrGrCrCrArGrUrArGrCrA
e14a2 fragment	rCrCrArCrUrGrGrArUrUrUrArArGrCrArGrArGrUrUrCrArA*rArArGrCrCrC
	rUrUrCrArGrCrGrGrCrCrArGrUrArGrCrA
e19a2 fragment	rCrArCrUrGrArArGrGrCrArGrCrCrUrUrCrGrArCrGrUrCrA*rArArGrCrCrC
	rUrUrCrArGrCrGrGrCrCrArGrUrArGrCrA
SLP-e13	/P/CTTCCTTATTGATGGTCAGCTTTATCGTCGTGACTGTTTGTAATAGG
	ACAGAGCCCCGCACTTTCAGTCACGACGAT
SLP-a2	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGATTTTCCTCT
	GCTGTCGTTTTACTGGCCGCTGAAGGGCTT
Universal primer 1 (UP1)	ATCGTCGTGACTGAAAGTGCGGGGCTCTGTCCTATTAC
Universal primer 2 (UP2)	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGA

The letter ‘r’ indicates ribonucleotides. The symbol ‘*’ indicates the fusion junction. The letter ‘P’ indicates a phosphate group.

2. Recycling amplification of LAMP

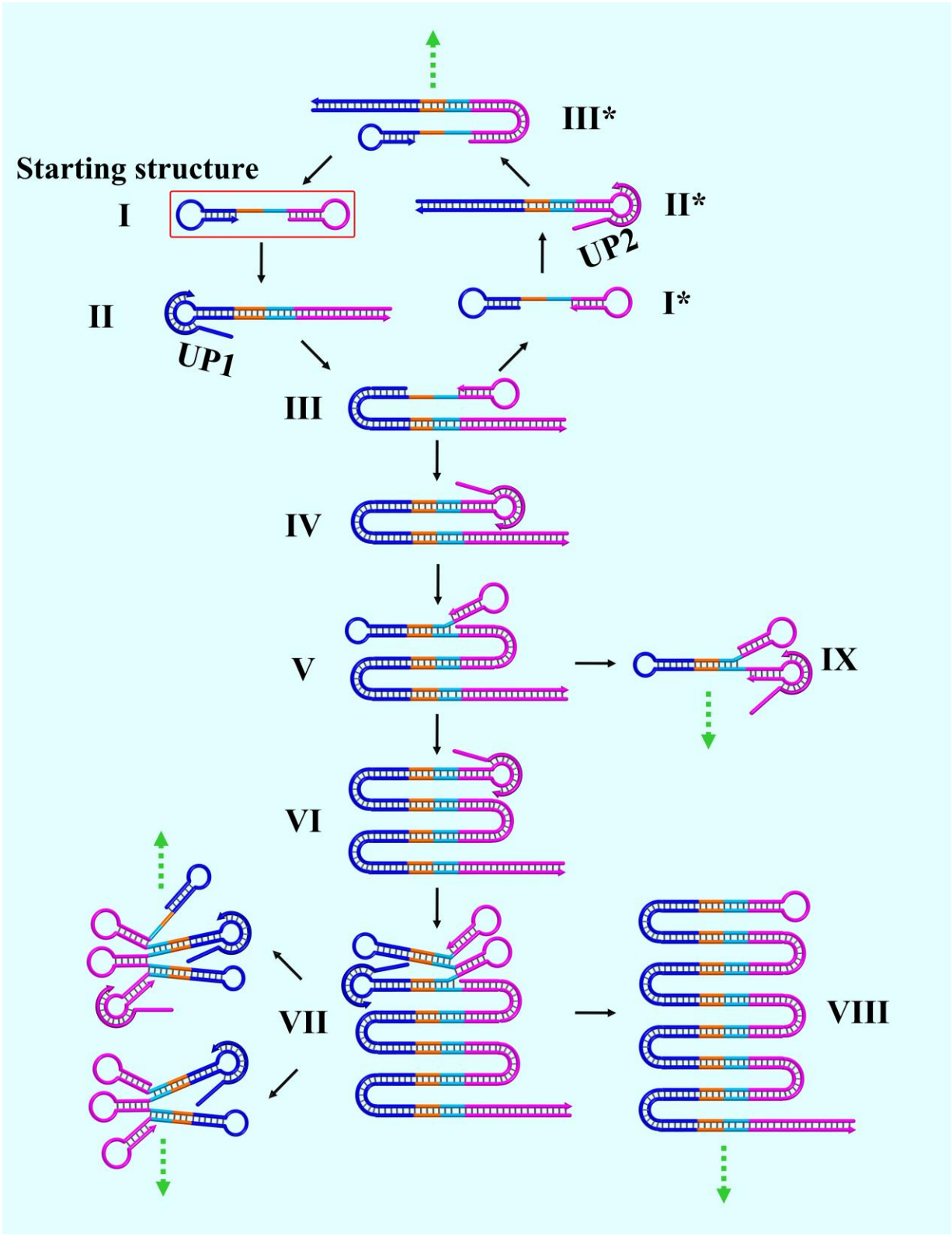

Figure S1. Recycling amplification of LAMP. The double stem-loop structure DNA (I) is the starting structure for the amplification.

The recycling amplification steps of the LAMP are illustrated in Figure S1. The ligated double stem-loop structure I can serve as the starting materials for the recycling amplification of LAMP. Once the ligated double stem-loop structure DNA is formed, it can auto-start the self-primed DNA synthesis to form structure II. Then primer UP1 hybridizes to the loop of structure II and primes strand displacement DNA synthesis to generate the structure III. Subsequent self-primed strand displacement DNA synthesis yields the structure IV and an anti-sequence (I*) of original double stem-loop structure I. The structure I* then produces structure II* by the self-primed DNA synthesis. Then primer UP2 hybridizes to the loop of the structure II* and primes strand displacement DNA synthesis to generate the structure III* which can generate newly double stem-loop structure I and another longer stem-loop structure DNA by self-primed strand displacement DNA synthesis. All newly formed structures (IV-XI) will initiate the recycling self-primed or primer UP1/UP2-primed strand displacement DNA synthesis. Therefore, the final products are a mixture of stem-loop structure DNAs with various stem lengths just like structure II, IV, VI, VIII, and longer stem-loop DNAs.

3. Optimization of the concentration of stem-loop DNA probes

The Vis-Fusion LIEXA method for fusion gene assay is based on the target-splinted ligation-triggered isothermal exponential amplification. So, conditions of ligation reaction and isothermal exponential amplification (IEXA) reaction will affect the performance of the Vis-Fusion LIEXA method for fusion gene analysis.

For ligation reaction, the concentration of stem-loop DNA probes and reaction time of ligation affect the efficiency and the specificity of the ligation reaction. Firstly, to investigate the influence of the concentration of stem-loop DNA probes for fusion gene assay, the 10 fM, 1 fM, 100 aM, and Blank (without any target) e13a2 target were simultaneously detected with the Vis-Fusion LIEXA method by using different concentration of stem-loop DNA probes (SLP-e13 and SLP-a2). As depicted in Figure S2A, when 20 pM stem-loop DNA probes were used, excepted color changes were produced by 10 fM and 1 fM e13a2 target but no color change was produced by 100 aM e13a2 target indicating that 100 aM target cannot be detected with the proposed method. In addition, on this condition, the normalized RGB values (G/(R+G+B)) will produce a larger deviation (error bars in Figure S2B) for smartphone-assisted quantifying analysis. When the concentration of stem-loop DNA probes was elevated to 200 pM, as low as 100 aM e13a2 target can be visually detected with the well-defined color change. When the concentration of stem-loop DNA probes further was elevated to 2 nM, the blank appeared undesired color change indicating that some non-template depended ligation occur on this condition. The non-template depended ligation will challenge the specificity and reliability of the Vis-Fusion LIEXA method. Therefore, taking into considerations of

high sensitivity, high specificity, and low deviation, 200 pM stem-loop DNA probes were selected for the Vis-Fusion LIEXA assay.

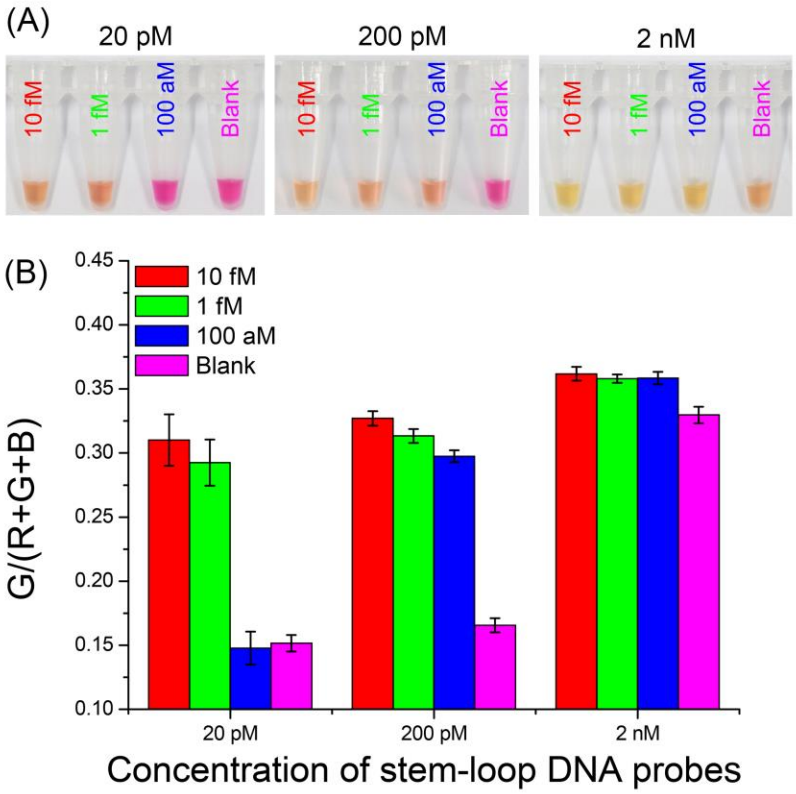

Figure S2. The influence of the concentration of stem-loop DNA probes for fusion gene assay by using Vis-Fusion LIEXA. (A) Colorimetric detection of 10 fM, 1 fM, 100 aM, 0 (Blank) e13a2 target with different concentrations of stem-loop DNA probes (SLP-e13 and SLP-a2). (B) The normalized RGB values of each reaction tube in Figure S2(A). The reaction time of ligation: 20 min. The reaction time of IEXA reaction: 20 min. Other experimental conditions are the same as described in the experimental protocols. Error bars were estimated from triplicate measurements.

4. Optimization of the reaction time of ligation

On the other hand, the effect of the reaction of ligation for the Vis-Fusion LIEXA-based fusion gene assay is also investigated. As displayed in Figure S3A, when the reaction time of ligation was 10 min, excepted color changes were produced by 10 fM and 1 fM e13a2 target but no color change was produced by 100 aM e13a2 target indicating that 100 aM target cannot be detected with the proposed method. In addition, on this condition, the normalized RGB values (G/(R+G+B)) will produce a larger deviation (error bars in Figure S3B) for smartphone-assisted quantifying analysis. When the reaction time of ligation was increased to 15 min, 20 min and 25 min, as low as 100 aM e13a2 target can be visually detected with the well-defined color change. In this regard, taking into considerations of high sensitivity and low deviation, 20 min was selected for the Vis-Fusion LIEXA assay.

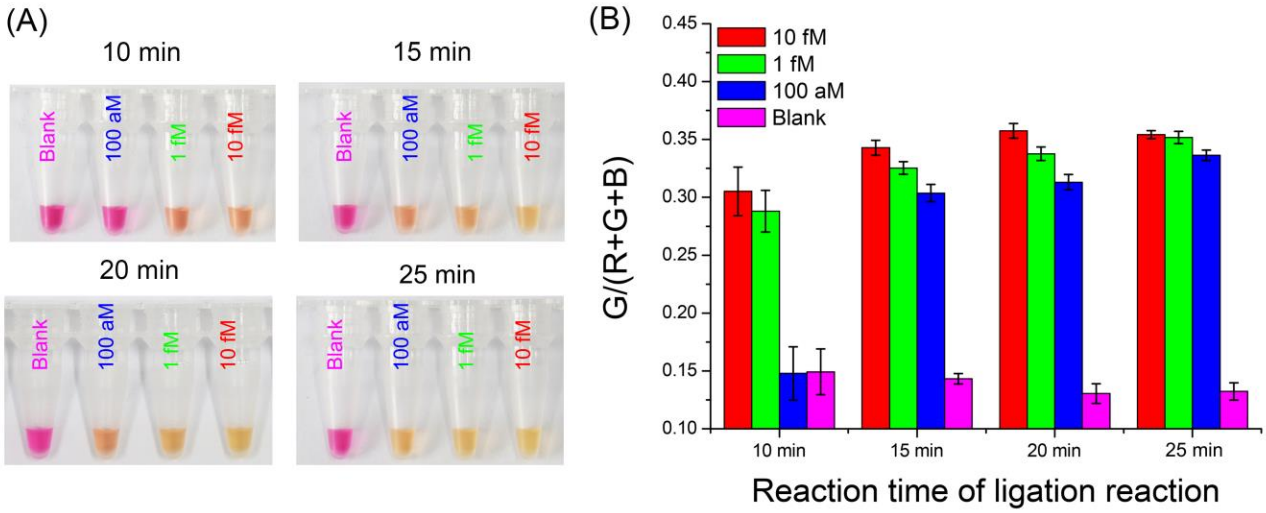

Figure S3. The influence of the reaction time of ligation for fusion gene assay by using Vis-Fusion LIEXA. (A) Colorimetric detection of 10 fM, 1 fM, 100 aM, 0 (Blank) e13a2 transcript with different reaction time of ligation (10 min, 15 min, 20 min, and 25 min). (B) Normalized RGB values of each reaction tube in Figure S4(A). Concentration of stem-loop DNA probes: 200 pM. Reaction time of IXEA reaction: 20 min. Other experimental conditions are the same as described in the experimental protocols. Error bars were estimated from triplicate measurements.

5. Optimization of the reaction time of IEXA reaction

During the rapid and efficient IEXA reaction, by-products including of pyrophosphates (PPi) and a hydrogen ion (H^+) that are released as each dNTP is incorporated into the nascent DNA. The H^+ released in the amplification will cause the pH drop resulting in the color change of cresol red, which can be used for fusion gene assay. The amount of released H^+ depends on the extent of IEXA reaction, which relies on the reaction time of IEXA. To obtain appropriate reaction time of IEXA for fusion gene assay, the Vis-Fusion LIEXA-based assay was used to detect 10-fold serial dilutions of a synthetic e13a2 fusion transcript fragment at different IEXA reaction time. As exhibited in Figure S4A, with the increasing of the reaction time of IEXA, the color changes after IEXA reaction of e13a2 positive tubes became more and more obvious. Meanwhile, with the increasing of the e13a2 fusion transcript concentration, the color changes after IEXA reaction became more obvious. When the reaction time of IEXA reaction is 16 min, as shown in Figure S4B, all the G/(R+G+B) values of e13a2 positive tubes can be well distinguished each other and blank. More importantly, the G/(R+G+B) values are linearly dependent on the logarithm (lg) of the target concentration in the ranges 100 pM-100 fM and 100 fM-100 aM (Figure 3 in the main text). To obtain a wide quantitative detection range for the fusion gene, 16 min was selected as the optimal reaction time of IEXA amplification.

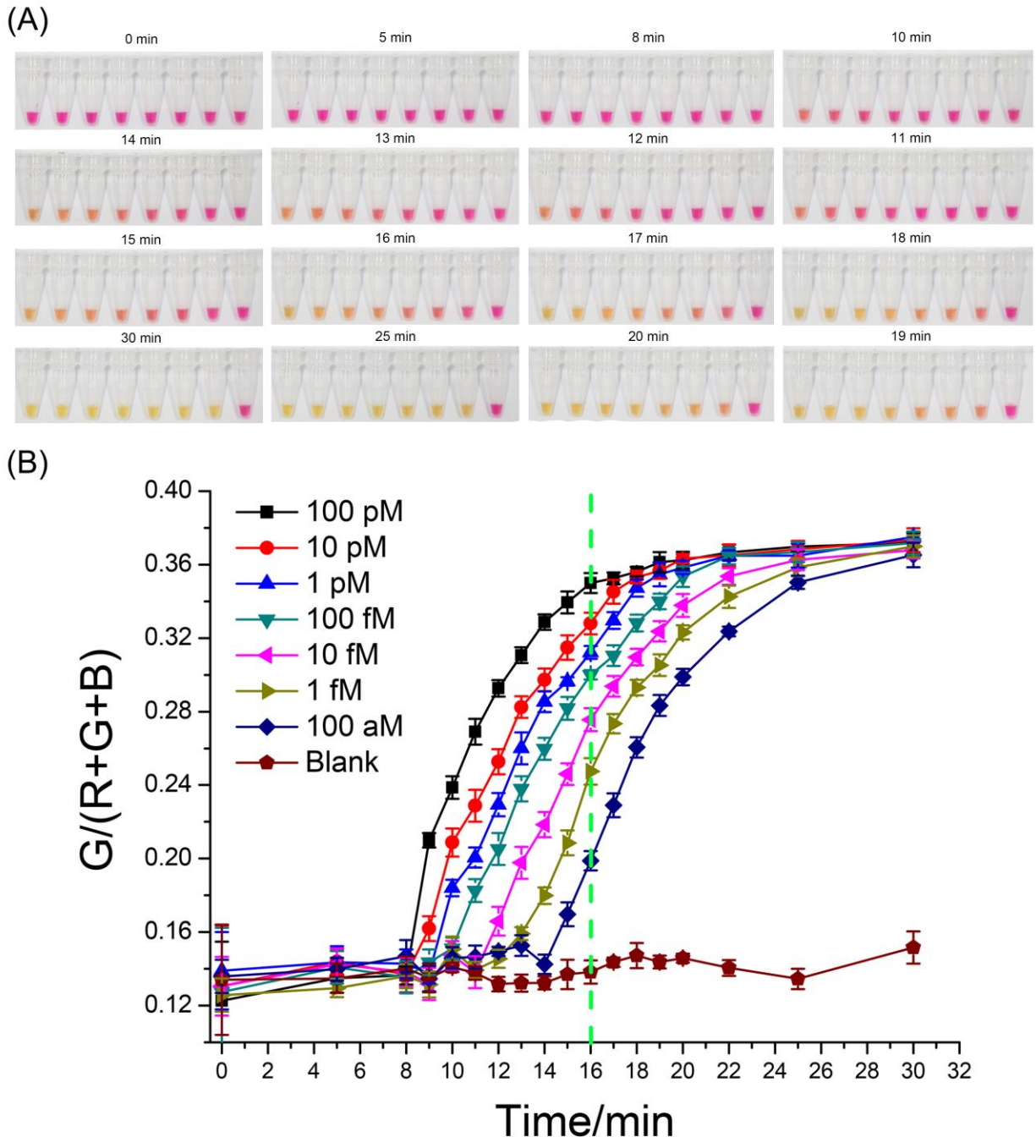

Figure S4. The effect of the reaction time of IEXA for fusion gene assay by using Vis-Fusion LIEXA. (A) Colorimetric detection of e13a2 transcript with different reaction time (shown on the top of each picture) of IEXA reaction. From left to right, the concentration of e13a2 is 100 pM, 10 pM, 1 pM, 100 fM, 10 fM, 1 fM, 100 aM and 0 (Blank), respectively. (B) Rate value of G/(R+G+B) of each reaction tube in Figure S4(A). Concentration of stem-loop DNA probes: 200 pM. Reaction time of ligation: 20 min. Other experimental conditions are the same as described in the experimental protocols. Error bars were estimated from triplicate measurements.