Electronic Supplementary Material (ESI) for RSC Advances.


# Electronic Supporting Information For:

## Real-time Detection of mRNA Splicing Variants with Specifically Designed Reverse-Transcription Loop-mediated Isothermal Amplification

Fengxia Su, Guanhao Wang, Jianing Ji, Pengbo Zhang, Fangfang Wang and Zhengping Li*

*School of Chemistry and Biology Engineering, University of Science and Technology Beijing, Beijing, 100083, P. R. China.*

*E-mail: lzpbd@ustb.edu.cn*

## List of contents:

1 Sequences of the DNA primers used in the experiments

Table S1 Sequences of DNA primers used in the experiments (5′-3′).

| DNA primers | Sequences (5′-3′) |
|---|---|
| IIIc-FIP | AGAGAACCTCAATCTCTTTGTCCGCCCTACCTCAAGGTTCTCAA |
| IIIc-BIP | TGAGGACGCTGGGGAATATACGGCAGAACTGTCAACCATG |
| IIIc-F3 | TAAATACGGGCCCGACG |
| IIIc-B3 | CTGTAATCTCCTTTTCTCTTCCA |
| IIIb-FIP | CCAGGTAGTCTGGGGAAGCTGTAACTCACTGTCCTGCCAAAACA G |
| IIIb-BIP | TGCATAGGGGTCTTCTTAATCGCCTGGTCGTGTTCTTCATTCGG |
| IIIb-F3 | CAGGCCAACCAGTCTGC |
| IIIb-B3 | GCTGGCTGCTGAAGTCTG |
| Reverse transcription primer | TGTTACCTGTCTCCGCAG |
| Forward primer | ACAGTGGTCGGAGGAGACGTAGAGTTTG |
| Reverse primer | GGGATACGTTTGGTCAGCTTGTGCACAG |

## 2 Sequences of FGFR 2-IIIc and FGFR 2-IIIb cloned in pUC57

**FGFR 2-IIIc (T7+GG+7-9-10)**

TAATACGACTCACTATAGGGGGAGCGATCGCCTCACCGGCCCATCCTCCAAGCCGGACTGCCGGCAAATGCCTCCACAGTGGTCGGAGGAGACGTAGAGTTTGTCTGCAAG

GTTTACAGTGATGCCCAGCCCCACATCCAGTGGATCAAGCACGTGGAAAAGAACGGCAGTAAATACGGGCCCGACGGGCTGCCCTACCTCAAGGTTCTCAAGGCCGCCGGTG

TTAACACCACGGACAAAGAGATTGAGGTTCTCTATATTCGGAATGTAACTTTTGAGGACGCTGGGGAATATACGTGCTTGGCGGGTAATTCTATTGGGATATCCTTTCACTCTGC

ATGGTTGACAGTTCTGCCAGCGCCTGGAAGAGAAAAGGAGATTACAGCTTCCCCAGACTACCTGGAGATAGCCATTTACTGCATAGGGGTCTTCTTAATCGCCTGTATGGTGGT

AACAGTCATCCTGTGCCGAATGAAGAACACGACCAAGAAGCCAGACTTCAGCAGCCAGCCGGCTGTGCACAAGCTGACCAAACGTATCCCCCTGCGGAGACAGGTAACA

RT-primer

**FGFR 2-IIIb (T7+GG+8-10)**

TAATACGACTCACTATAGGGGGCACTCGGGGATAAATAGTTCCAATGCAGAAGTGCTGGCTCTGTTCAATGTGACCGAGGCGGATGCTGGGGAATATATATGTA

AGGTCTCCAATTATATAGGGCAGGCCAACCAGTCTGCCTGGCTCACTGTCCTGCCAAAACAGCAAGCGCCTGGAAGAGAAAAGGAGATTACAGCTTCCCCAGACTAC

CTGGAGATAGCCATTTACTGCATAGGGGTCTTCTTAATCGCCTGTATGGTGGTAACAGTCATCCTGTGCCGAATGAAGAACACGACCAAGAAGCCAGACTTCAGCAGCCAGCC

GGCTGTGCACAAGCTGACCAAACGTATCCCCCTGCGGAGACAGGTAACA

RT-primer

Fig. S1 Sequences of FGFR 2-IIIc and FGFR 2-IIIb cloned in pUC57.

3 Agarose electrophoresis images of constructed plasmids of FGFR 2-IIIc and FGFR 2-IIIb

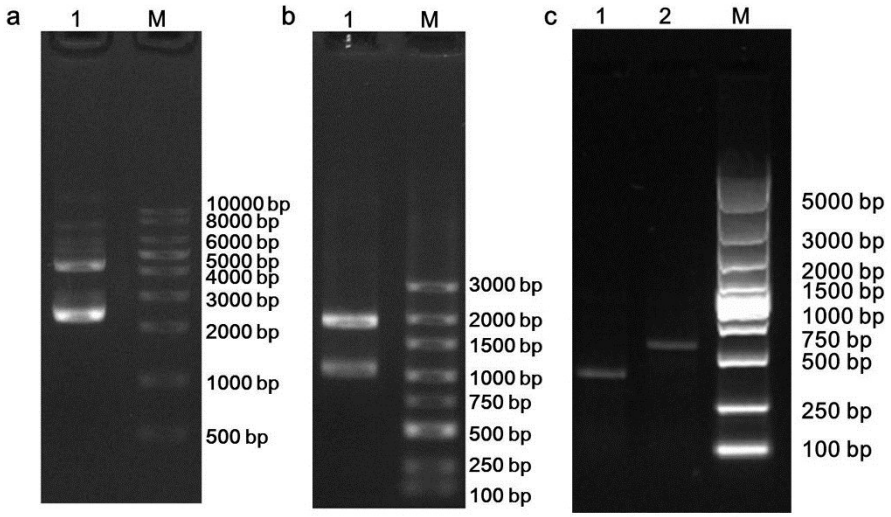



Fig. S2 Agarose electrophoresis images of constructed plasmids of FGFR 2-IIIc (a) and FGFR 2-IIIb (b). (c) From left to right, purified FGFR 2-IIIb and FGFR 2-IIIc and DNA marker.

## 4 Effect of the amount of Bst 2.0 WarmStart DNA polymerase on FGFR 2-IIIc assay

DNA polymerase is the catalyst of LAMP reaction. Accordingly, the optimal amount of Bst 2.0 WarmStart DNA polymerase was investigated for FGFR 2-IIIc assay. 1.6, 2.4 and 3.2 U of Bst 2.0 WarmStart DNA polymerase were used to catalyze 0 (blank), 1 fM, 10 fM and 100 fM FGFR 2-IIIc, respectively, to perform the proposed assay. As shown in Fig. S3, 1.6 U Bst 2.0 WarmStart DNA polymerase was insufficient to catalyze the efficient amplification of 1 fM FGFR 2-IIIc within 60 min. When the amount of DNA polymerase increased to 2.4 U and 3.2 U, the reaction was obviously accelerated. However, the fluorescence curves between targets of different concentrations catalyzed by 3.2 U Bst 2.0 WarmStart DNA polymerase were narrower than that by 2.4 U Bst 2.0 WarmStart DNA polymerase. 2.4 U Bst 2.0 WarmStart DNA polymerase can not only catalyze the reaction to detect 1 fM target, but also have a better distinguishing effect on different concentrations of FGFR 2-IIIc. Therefore, 2.4 U was selected as the final dosage of the LAMP-based method for FGFR 2-IIIc detection.

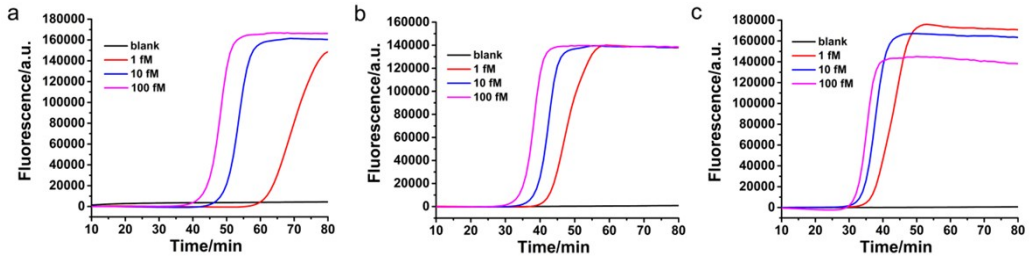



Fig. S3 Effect of the amount of Bst 2.0 WarmStart DNA polymerase on FGFR 2-IIIc assay. The real-time fluorescence curves were produced from blank, 1 fM, 10 fM and 100 fM FGFR 2-IIIc with the LAMP-based method by adding (a) 1.6 U (b) 2.4 U (c) 3.2 U Bst 2.0 WarmStart DNA polymerase. Other experimental conditions were consistent with those in the standard protocols.

## 5 Influence of the concentration of IIIc-FIP/BIP/F3/B3 on the FGFR 2-IIIc assay

DNA primers are the raw materials of LAMP amplification, the concentration of which is a critical factor for optimization. The ratio of the four primers (IIIc-FIP, IIIc-BIP, IIIc-F3, IIIc-B3) is fixed at 8:8:1:1, but the concentration of primers was indicated as the concentration of IIIc-FIP. When 1.2 μM primers were employed, 1 fM, 10 fM and 100 fM FGFR 2-IIIc could produce well-defined real-time fluorescence curves. However, 100 aM of FGFR 2-IIIc exhibited no fluorescence increase during 80 min (Fig. S4), indicating the low concentration of primers led to low efficiency of LAMP reaction, especially at low concentration of target. When the concentration of DNA primers was increased to 1.6 μM and 2.0 μM, 100 aM of target could be detected and the POI values of the fluorescence curves decreased. However, the spacing between the fluorescence curves by using 2.0 μM primers became smaller, meaning the amount of DNA primers were excessive to differentiate different concentrations of targets. Hence, 1.6 μM was chosen as the optimal concentration of DNA primers for the LAMP-based method for FGFR 2-IIIc detection.

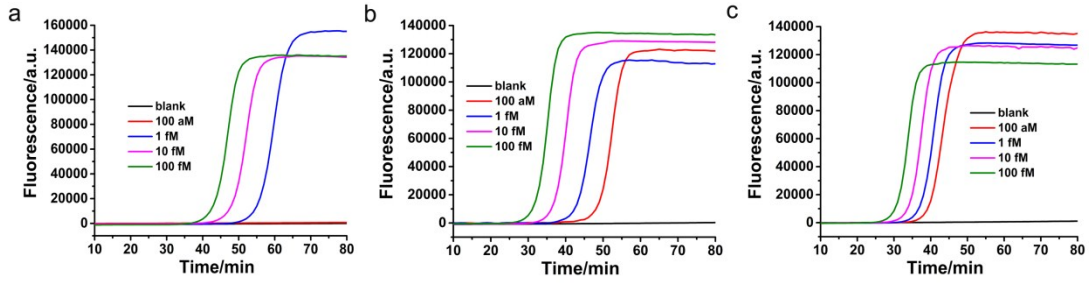



Fig. S4 Influence of the concentration of IIIc-FIP/BIP/F3/B3 on the FGFR 2-IIIc assay. The real-time fluorescence curves were produced from blank, 100 aM, 1 fM, 10 fM and 100 fM of FGFR 2-IIIc by changing the concentrations of primers as (a) 1.2 μM (b) 1.6 μM (c) 2.0 μM. Other experimental conditions were consistent with those in the standard protocols.

## 6 Impact of the amount of Bst 2.0 WarmStart DNA polymerase on the FGFR 2-IIIb assay

The optimal amount of Bst 2.0 WarmStart DNA Polymerase in FGFR 2-IIIb detection was also investigated. 0, 1 fM, 10 fM and 100 fM of FGFR 2-IIIb were catalyzed by DNA polymerase ranging from 0.2 U to 1.6 U. As shown in Fig. S5, almost no specific amplification was observed within 50 min under the catalysis of 0.2 U DNA polymerase, indicating that the amount of DNA polymerase was too low to generate DNA amplification. Well-defined fluorescence curves were produced in presence of 0.8 U DNA polymerase and the POI values reduced as the amount of DNA polymerase increasing. When the amount of DNA polymerase increased to 1.6 U, the POI value of the fluorescence curves further reduced and fluorescence curves of 10 fM and 1 fM coincided, suggesting 1.6 U was excessive to catalyze the LAMP reaction. Accordingly, 0.8 U Bst 2.0 WarmStart DNA polymerase was employed to catalyze the LAMP reaction for FGFR 2-IIIb assay.

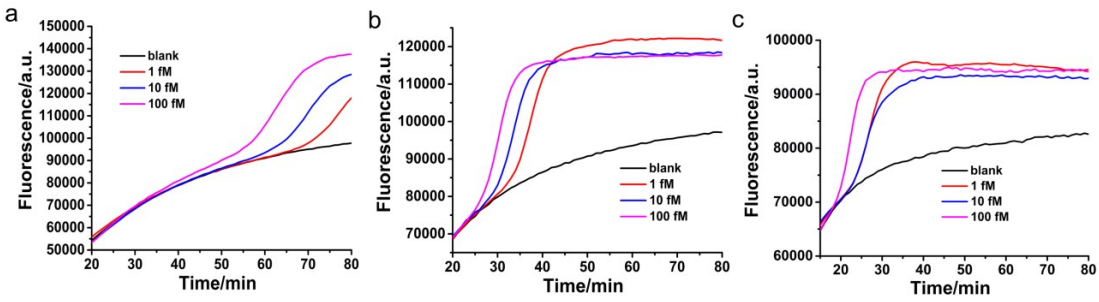
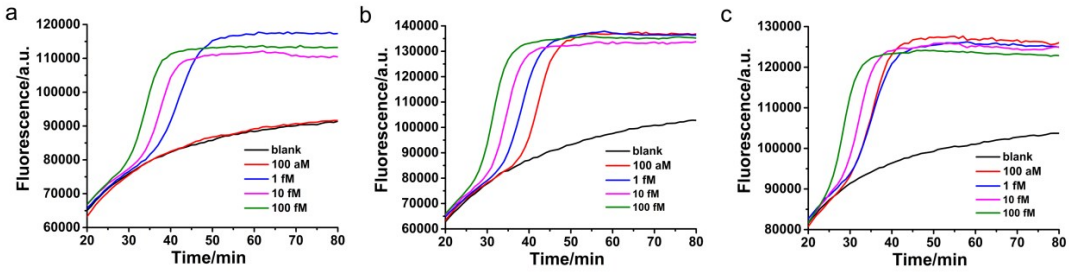



Fig. S5 Impact of the amount of Bst 2.0 WarmStart DNA polymerase on the FGFR 2-IIIb assay. The real-time fluorescence curves were produced from blank, 1 fM, 10 fM and 100 fM of FGFR 2-IIIb catalyzed with 0.2 U (a), 0.8 U (b) and 1.6 U (c) Bst 2.0 WarmStart DNA polymerase. Other experimental conditions were consistent with those in the standard protocols.

## 7 Influence of the concentration of IIIb-FIP/BIP/F3/B3 on the FGFR 2-IIIb assay

The concentration of four primers (FIP, BIP, F3, B3) was a key factor for the amplification efficiency of LAMP, thus it was optimized. The ratio of the four primers of IIIb-FIP, IIIb-BIP, IIIb-F3, IIIb-B3 was fixed at 8:8:1:1, and the concentration of primers was indicated as the concentration of IIIb-FIP. As shown in Fig. S6, when 0.8 μM primers were used, 100 aM target could not be detected by the proposed method, indicating it is an insufficient amount of primers. Well-defined fluorescence curves were obtained from 100 aM to100 fM FGFR 2-IIIb with 1.2 μM DNA primers. With further increasing the concentration of primers to 1.6 μM, the POI value of the fluorescence curves reduced and 1 fM target could not be discriminated with 100 aM target, suggesting 1.6 μM primers was excessive for the detection of FGFR 2-IIIb. Accordingly, 1.2 μM was selected as the final primer concentration for FGFR 2-IIIb detection.




Fgiure S6 Influence of the concentration of IIIb-FIP/BIP/F3/B3 on the FGFR 2-IIIb assay. The LAMP-based assay and real-time fluorescence measurement were originated from blank, 100 aM, 1 fM, 10 fM and 100 fM of FGFR 2-IIIb with different IIIb-FIP/BIP/F3/B3 concentrations of 0.8 μM (a), 1.2 μM (b) and 1.6 μM (c). Other experimental conditions were consistent with those in the standard protocols.

# 8 Sequencing results of FGFR 2 mRNA from cells

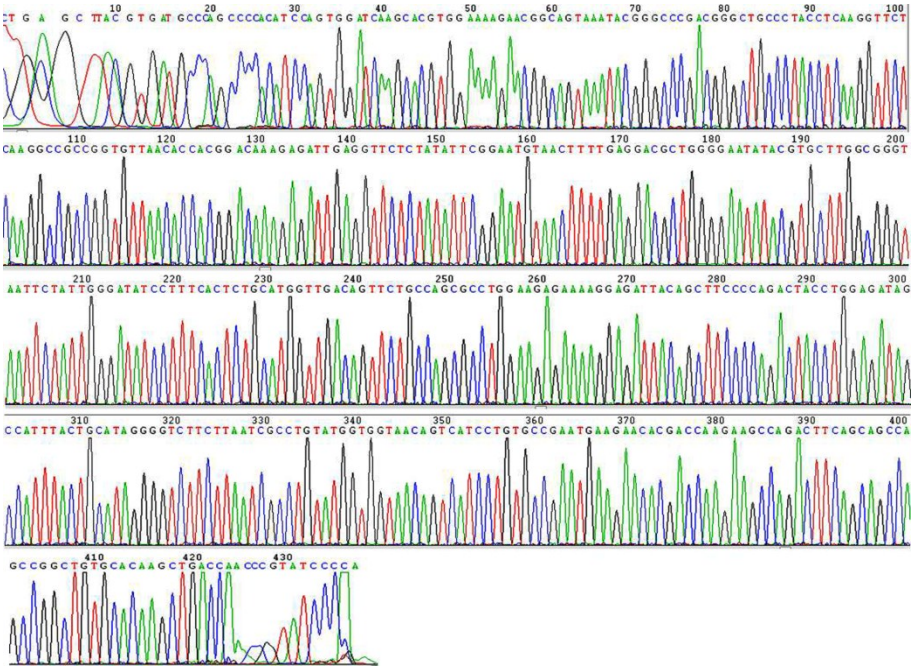


Fig. S7 Sequencing result of the exon 7 to exon 10 of FGFR 2 mRNA from A549 cells.

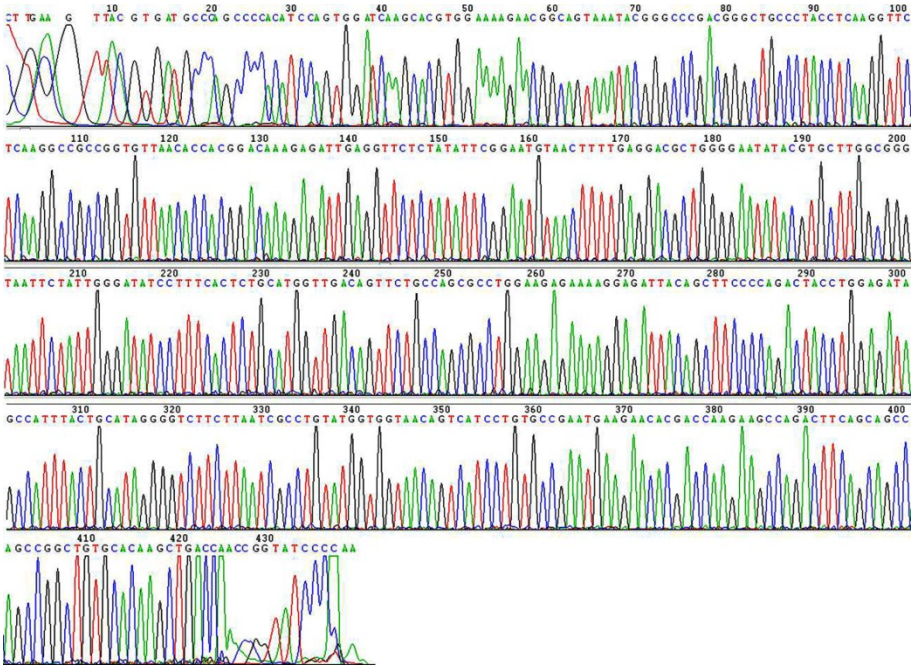


Fig. S8 Sequencing result of the exon 7 to exon 10 of FGFR 2 mRNA from 293T cells.