

Electronic Supporting information

Specific detection of fusion transcripts based on duplex-specific nuclease and isothermal exponential amplification reaction

Fengxia Su, Tian Li, Xiaofei He, Zhengping Li*

Beijing Key Laboratory for Bioengineering and Sensing Technology; School of
Chemistry and Biological Engineering, University of Science and Technology Beijing,
30 Xueyuan Road, Haidian District, Beijing, China, 100083

E-mail: lzpbd@ustb.edu.cn

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1. Table S1 Sequences of RNA targets and DNA oligonucleotides used in this work.

Name	Sequences (5' -3')
e1a2	GAGGGCGCCUCCAUGGAGACGCAGAAGCCCUUCAGCG GCCAGUAGCAUCU
e13a2	AGCAUCCGCUGACCAUCAAUAAAGGAAGAAGCCCUUCA GCGGCCAGUAGCA
e1a2-probe	TACCATCAAGCGCCAGCTGAAGGGCTTCTGCGTCTCCA
e13a2-probe	TACCATCAAGCGCCAGCTGAAGGGCTTCTTCCTTATTGA TGG
Tem6	CTGGCGCTTGATGGTAAACAGACTCCTTCAGCTGGCGCT TGATGGTA-PO₄
Tem9	CTGGCGCTTGATGGTAAACAGACTCGCCCTTCAGCTGG CGCTTGATGGTA-PO₄
Tem11	CTGGCGCTTGATGGTAAACAGACTCAAGCCCTTCAGCTG GCGCTTGATGGTA-PO₄

Note: Red and purple in e1a2 target represent the sequences originated from BCR and ABL gene, respectively. Green and purple of e13a2 target represent the sequences from BCR and ABL gene, respectively. In e1a2-probe and e13a2-probe, the sequences complementary to specific target are indicated with the same colors with the specific target accordingly. The blue sequences in e1a2-probe and e13a2-probe are complementary with the blue sequences in Tem6, Tem9 and Tem11.

2. The construction of BCR-ABL fusion transcripts e1a2 and e13a2.

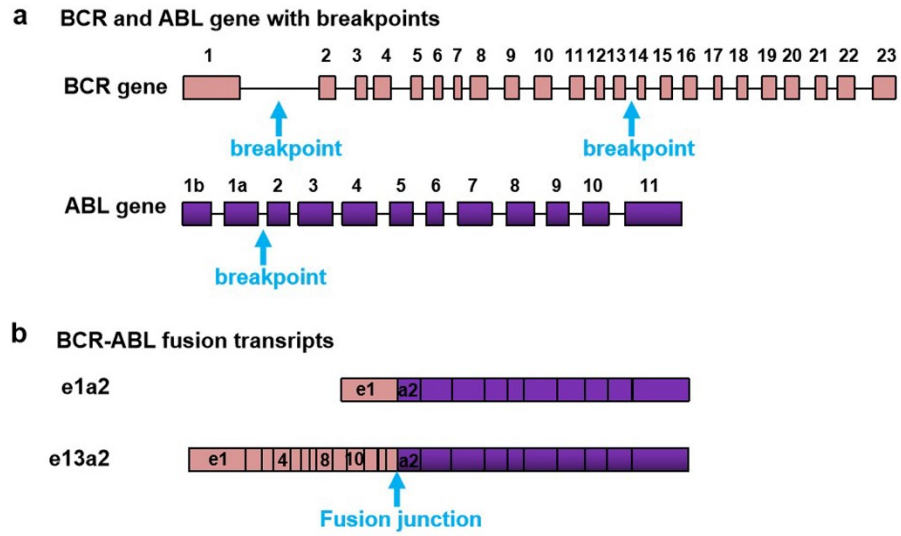


Fig. S1. (a) BCR and ABL genes with breakpoints. The numbers indicate the exons. The black line between exons represent introns. (b) The construction of e1a2 and e13a2 fusion transcripts. Arrow indicates the fusion junction.

3. The optimization of the amount of vent (exo-) DNA polymerase

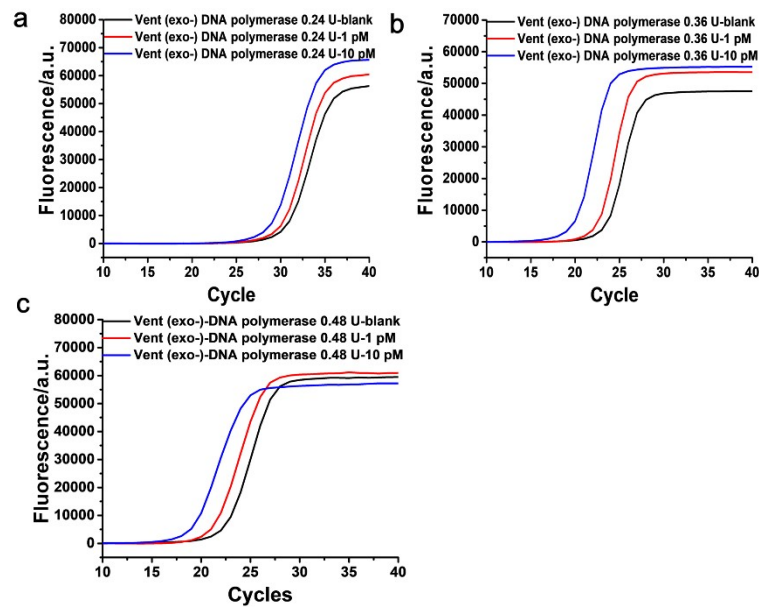


Fig. S2. Influence of the amount of the vent (exo-) DNA polymerase on the detection of e1a2 target by the proposed method. (a) 0.24 U, (b) 0.36 U and (c) 0.48 U were used in the proposed assays and the concentrations of e1a2 target were 0 (blank), 1 pM and 10 pM, respectively, under each condition.

Vent (exo-) DNA polymerase is employed to catalyze the DNA synthesis and DNA displacement in the IEXPAR and has close relationship with the reaction rate of IEXPAR. Thus, the amount of vent (exo-) DNA polymerase in the range from 0.24 U to 0.48 U were investigated by using e1a2 as model target. As shown in Fig. S2a, when 0.24 vent (exo-) DNA polymerase is used, the rate of IEXPAR is slow and the fluorescence curve produced from 10 pM e1a2 is close to that of 1 pM e1a2, indicating the amount of the vent (exo-) DNA polymerase is insufficient to catalyze the reaction. With the amount of vent (exo-) DNA polymerase increasing to 0.36 U (Fig. S2b), the POI value of three curves reduce and the fluorescence curve of 10 pM e1a2 can be well distinguished from that of 1 pM e1a2 and blank, indicating that the increased DNA polymerase elevates the specific amplification rate. When continued to increase the vent (exo-) DNA polymerase dosage to 0.48 U (Fig. S2c), the reaction rate of the blank sample is accelerated more, so the usage of the DNA polymerases is over dose for the effective detection. Therefore, 0.36 U vent (exo-) DNA polymerase is optimal in the DSN-IEXPAR assay.

4. The optimization of the amount of Nt.BstNBI

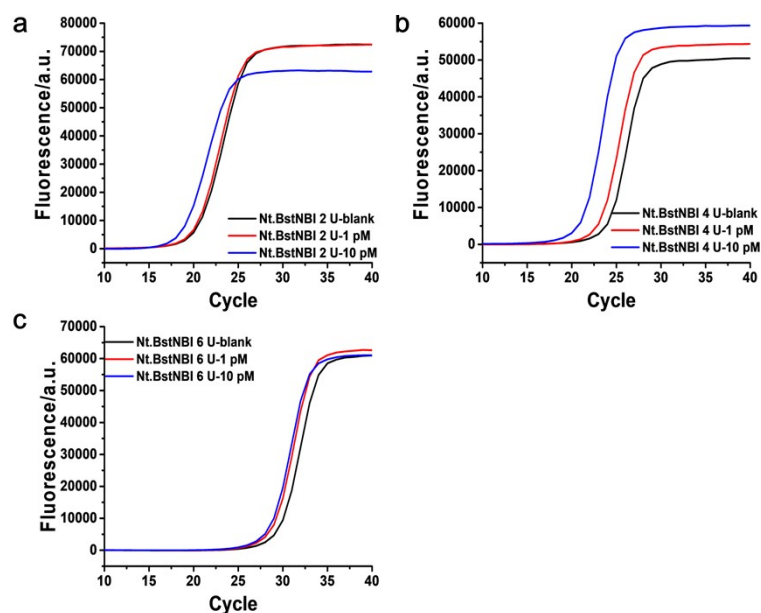


Fig. S3. Effect of the amount of the Nt.BstNBI on the detection of e1a2 target by using the proposed method. (a) 2 U, (b) 4 U and (c) 6 U were used in these assays and the concentrations of e1a2 target were 0, 1 pM and 10 pM, respectively, under each condition.

According to the principle of DSN-IEXPAR, Nt.BstNBI, as a nicking enzyme has important role, so the amount of Nt.BstNBI was also investigated. When 2 U Nt.BstNBI was used (Fig. S3a), the fluorescence curve of 10 pM is close to that of 1 pM e1a2, which is the same with blank response. As increasing the amount of Nt.BstNBI to 4 U (Fig. S3b), the fluorescence curves 10 pM and 1 pM e1a2 can be well discriminated from blank. When the dosages of Nt.BstNBI increases to 6 U (Fig. S3c), the POI value of the fluorescence curves increases and the curves are closely, indicating that the usage is excessive for the reaction and results in cleavage before extension. Accordingly, 4 U is selected as the optimum dose for Nt.BstNBI.

5. The optimization of the amount of DSN

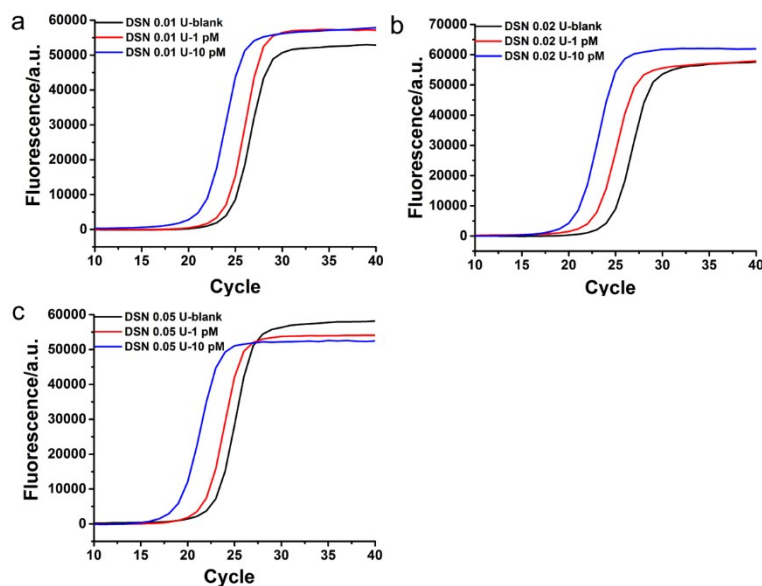


Fig. S4. Influence of the amount of the DSN on the detection of e1a2 target by using the proposed method. (a) 0.01 U, (b) 0.02 U and (c) 0.05 U were used in these assays and the concentrations of e1a2 target were 0, 1 pM and 10 pM, respectively, under each condition.

DSN can specifically recognize and cut the DNA in the duplex of fusion transcript and DNA probe, and the released DNA fragment can subsequently initiate IEXPAR. The amount of DSN is investigated for the DSN-IEXPAR assay. When 0.01 U DSN is used (Fig. S4a), the fluorescence curve of 1 pM e1a2 is close to that of blank, indicating the amount of DSN is slightly low to cut the DNA. With the amount of DSN increases to 0.02 U (Fig. S4b), the specific cutting reaction obviously elevates more than the blank. When continued to increase the DSN usage to 0.05 U (Fig. S4c), the POI value of fluorescence curve produced from blank sample decreases more than that of 1 pM e1a2, pulling down the discrimination among the three samples. Therefore, 0.02 U DSN is employed for the following assay.