Electronic Supplementary Information (ESI)

Isothermal amplification system based on template-dependent extension

1. Experimental Section

The molecular beacon (Takara Biotechnology Co., Ltd. Dalian, China) and other oligonucleotides (Invitrogen Bio Inc. Shanghai, China) were diluted to 50 μM in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) as stock solutions, and their sequences are shown in Table 1. 25 μL of amplification reactions contained molecular beacon (1 μM, 5 μL), assistant probe (1 μM, 5 μL), target DNA (5 μL), 400 μM dNTPs, 2 U Klenow Fragment (3'-5' exo$^-$) and 4 U Nt. BbvCI nicking endonuclease (New England Biolabs) in reaction buffer (20 mM Tris-Ac, 50 mM KAc, 10 mM $Mg(Ac)_2$, 1 mM DTT, pH 7.9). Reactions were assembled at 16 ℃ for 20 min and initiated by adding polymerase for another 20 min and nicking endonuclease then incubating the reaction at 37 ℃ for 2 h. Fluorescence measurements were performed by using a Hitachi F-7000 Fuorescence Spectrometer (Hitachi. Ltd., Japan) with an optical path length of 1.0 cm. The excitation was made at 494 nm and the emission spectra from 500 to 600 nm were collected with both excitations and emission lit set at 5.0 nm. Before fluorescence measuring, additional reaction buffer was added to make the resulting solution involved 100 μL.

The gel electrophoresis was carried out on 5% agarose (Biowest Agarose，Spain) gel at 100 V for 70 min in 0.5×TBE buffer (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA, pH 8.0) and visualized under UV light. The loading sample (15 μL) was prepared as above mentioned, but with following DNA concentration: molecular beacon (10 μM, 1.5 μL), assistant probe2 (10 μM, 1.5 μL), target DNA1 (1.5 μL). Low molecular weight DNA ladder (Fermentas) was incubated with SB Green for 10 min before used in this analysis.

2. Tables and Figures

Table 1. Oligonucleotides designed in the present study [a].

Note including abbreviation	Sequence (5'-3')
Molecular beacon (MB)	FAM-ACAGAG *ACACAAACACGCA* CTC**TGT**(Dabcyl) **GG**
Assistant probe1 (A1)	CATCATGCCCTCAGC**CCACA**CTCAAAGCTGTTCCGT AAAA
Assistant probe2 (A2)	CATCATGCCCTCAGC**CCACA**CTCAAAGCTGTTCC AAAA
Assistant probe3 (A3)	CATCATGCCCTCAGC**CCACA**CTCAAAGCTGTT AAAAA
Assistant probe4 (A4)	CATCATGCCCTCAGC**CCACA**CTCAAAGCTG AAAAA
Assistant probe5 (A5)	CATCATGCCCTCAGC**CCACA**CTCAAAGCT AAAAA
Assistant probe6 (A6)	CATCATGCCCTCAGC**CCACA**CTCAAAGC AAAAA
Target DNA0 (T0)	ACGGAACAGCTTTGAG *GTGCGTGTTTGTGCC* TGTC
Target DNA1 (T1)	ACGGAACAGCTTTGAG *GTGCGTGTTTG*AACC TGTC
Target DNA2 (T2)	ACGGAACAGCTTTGAG *GTGCGTGTTT*AAACC TGTC
Target DNA3 (T3)	ACGGAACAGCTTTGAG *GTGCGTGT*AAAAACC TGTC
Target DNA4 (T4)	ACGGAACAGCTTTGAG GA*GTGCGTGTTTGTG*CC TGTC
SNP1	ACGGAACAGCT TTGAG GTGC**A** TGTTTGAACC TGTC
SNP2	ACGGAACAGCT TTGAG GT**A**CGTGTTTGAACC TGTC
SNP3	ACGGAACAGCT TTGAG GTGCG**A**GTTTGAACC TGTC
SNP4	ACGGAACAGCT TTGAG GTGCGT**A** TTTGAACC TGTC

[a] For the MB, the quencher is conjugated to the underlined bold 'T'; the base fragments with grey backgrounds denote the complementary base sequences to form hairpin structure. MB can recognize the target DNA via the hybridization between their italics fragments. In assistant probe, bases highlighted in bold are complementary to MB, while underlined sequences are complementary to target DNA. A half recognition site for Nt.BbvCI nickase is highlighted in blue. The SNP site is highlighted in red. The sequence of T0 is a fragment of human p53 gene (exon 8).

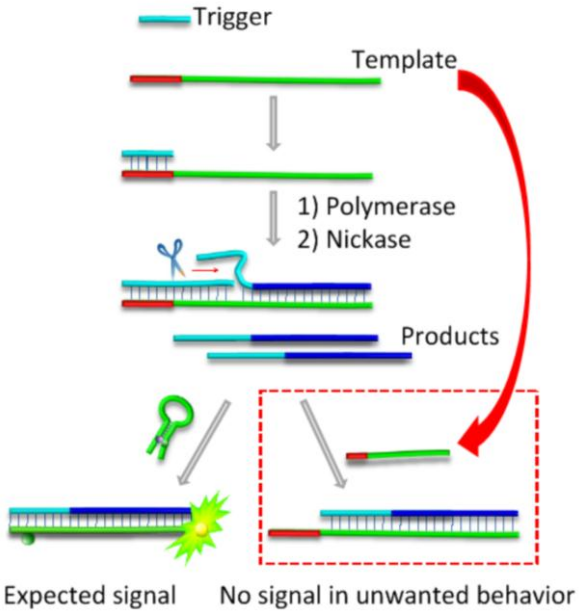

Fig. S1 Illustration of unwanted hybridization behavior in the SDA system.

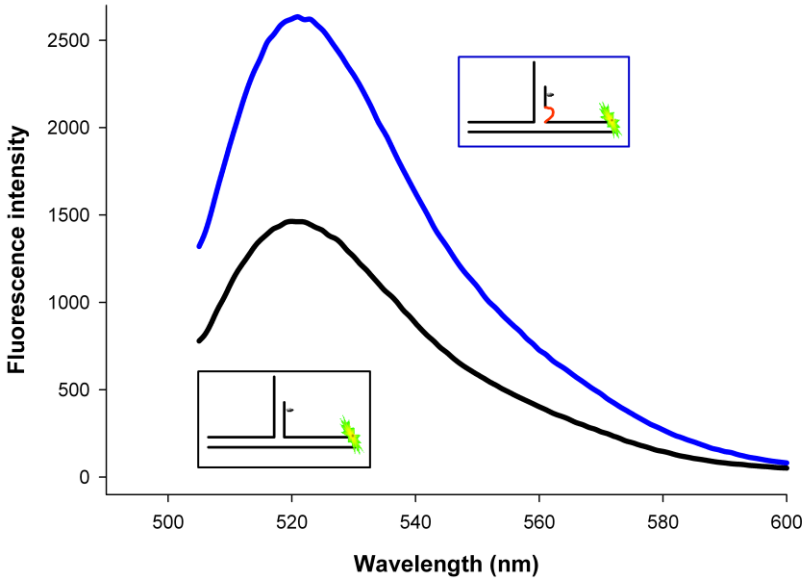

Fig. S2 Fluorescence emission spectra of amplified system with (bule line) and without (black line) bulge. As shown in top right corner, MB with "CT" bases in the middle region would form an unpaired nucleotide bulge when A2 and T0 are involved. When T4 is used to instead of T0, amplified system acts as a no-bulge model as shown in bottom left corner. Amplified system contains MB, A2, dNTPs, 2 U polymerase and 4 U nickase.

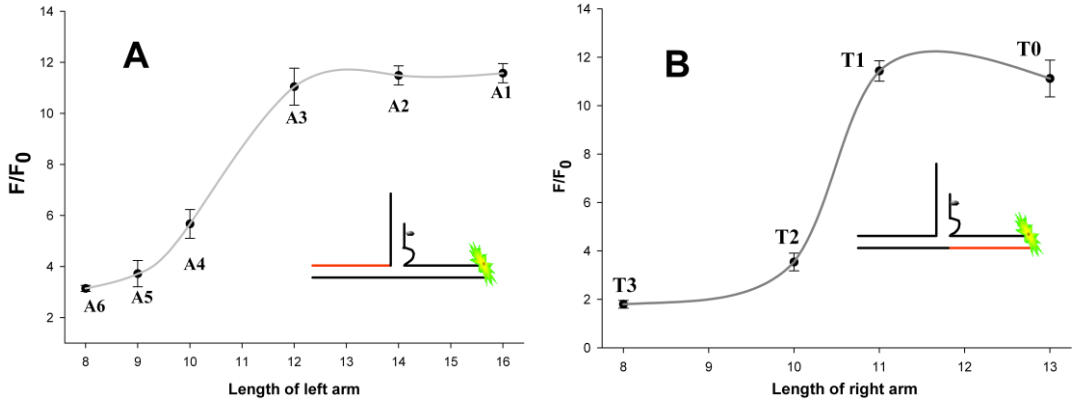

Fig. S3 The effect of the length of left arm (A) and the length of right arm (B) on the fluorescence response of the sensing system. T1 is used in (A), and A2 is adopted in (B). The descriptors F and F_0 are the fluorescence intensity of the sensing system at 519 nm in the presence and absence of 50 nM of target DNA, respectively. The average relative standard deviation of all data points were obtained by measuring each sample at least three times. Left arm stands for the hybridized base pairs between assistant probe and target DNA, while right arm stands for the base pairs resulting from the hybridization of MB to target DNA.

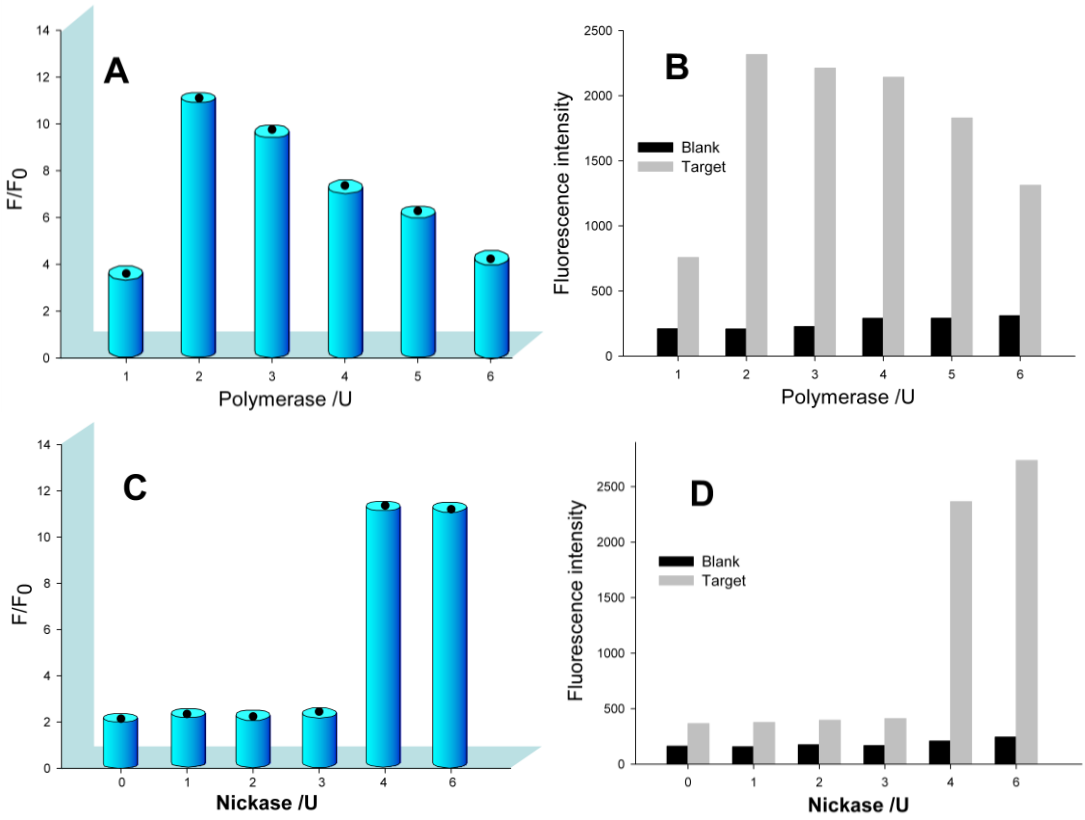

Fig. S4 The effect of A) the polymerase concentration and C) the nickase concentrationon the fluorescence response of the sensing system. The descriptors F and F_0 are the fluorescence intensity of the sensing system at 519 nm in the presence and absence of 50 nM of target DNA1, respectively. Assistant probe used in this section is A2. (B) and (D) are the background fluorescence values corresponding to (A) and (C), respectively. The amount of nickase and polymerase used in (A, B) and (C, D) are kept at 4 U and 2 U, respectively.

The concentrations of polymerase and nickase are also critical for the sensing performance. To estimate the importance of them, we incubated 200 nM MB and 200 nM assistant probe A2 with target DNA1 (0 or 50 nM) and then monitored the fluorescence change for the mixed solution with different concentrations of polymerase and nickase, respectively. The signal-to-background ratio results and the corresponding background signals are shown in Fig S4. Experimental results indicate that 2U of polymerase and 4U of nickase could provide a maximum S/N ratio for the sensing system. Additionally, we observed that the fluorescence enhancement is mainly induced by the addition of target DNA. Because the background signals with

the different concentrations of polymerase and nickase are kept in low values (see Fig S4B and D) which are close to the one of traditional system without adding any enzymes (see Fig 1 c). These results also indicate that non-specific background amplification has been largely avoided in our TEIA-based assay.

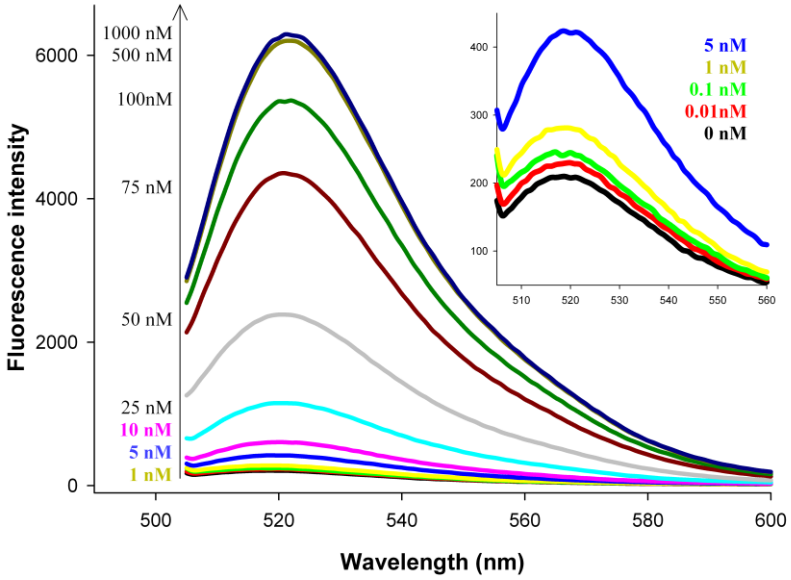

Fig. S5 Fluorescence spectra of amplified system at different concentrations of T1. Inset: response of the amplified system to T1 at a low concentration. Amplification system is composed of MB, A2, dNTPs, 2 U polymerase and 4 U nickase.

Fig. S5 shows fluorescence response to varying concentrations of target T1 in TEIA system. The increase in fluorescence peak intensity is observed with increasing target concentrations within about five-decade range from 0.01 to 500 nM. The dynamic response range is obviously wider than the three-decade range from 5 nM to 5000 nM in traditional system where polymerase and nickase are not involved (see Fig. 3). Excitingly, the signal induced by 0.01 nM T1 can still be detected, indicating a remarkable improvement in assay sensitivity compared with 5 nM detection limit of traditional system. Moreover, the fluorescence intensity at any datum point is higher than traditional system. For example, the fluorescence peak corresponding to 50 nM target T1 is about 2343±4.6%, while a much lower fluorescence peak, about 324± 4.4%, is detected at this target concentration in traditional system. Those results indicate that the developed TEIA system can induce the signal amplification for the target DNA detection. Additionally, RSDs of F/F_0 in TEIA system are 4.9%, 2.8%, 6.8%, 9.5% and 4.6% in at least three repetitive eassays of 0.01 nM, 0.10 nM, 1.0 nM, 5.0 nM and 100 nM T1(see Fig. 3). The average RSD is about 5.7%, and the highest one is not more than 10%, demonstrating a good reproducibility.

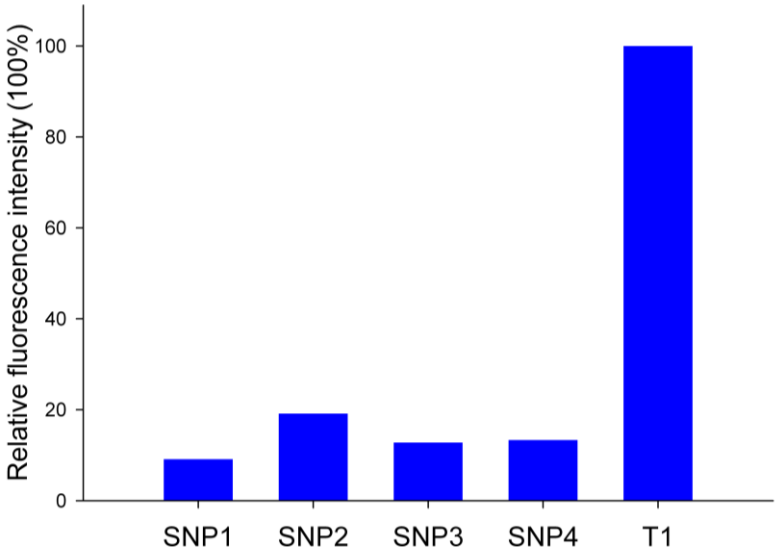

Fig. S6 Quantitative evaluation of the SNP discrimination ability in amplified system consisting of MB, A2, dNTPs, 2 U polymerase and 4 U nickase. The concentration of SNP DNAs and T1 are 50 nM. In this section, the change of fluorescence intensity induced by adding of T1 sample is defined as 100%, blank as 0%.