Supporting Information

Hybridization-triggered Isothermal Signal Amplification Coupled with MutS for Label-free and Sensitive Fluorescent Assay of SNPs

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Experimental Section:

Reagents and Instruments. All oligonucleotides (Table S1) were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China). The dNTP mixture (dNTPs), Klenow Fragment polymerase (KF^- polymerase) and MutS were purchased from New England Biolabs Inc (USA). SYBR Green I (SG, 20 × concentrate) was supplied by Generay Biotech Co., Ltd. (Shanghai, China). Other chemicals were of analytical grade and were used without further purification. Deionized water was obtained through Millpore water purification system.

Fluorescence Intensity Detection of Conformational Change of P_0. Before adding T_1, the P_0 was performed in 500 μL 20 mM Tris-buffer solution (pH 8.0) containing 100 mM NaCl, 5 mM $MgCl_2$. Following the additions of T_1, the solution was incubated at room temperature for 30 min and then the fluorescence intensity was recorded on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ). Fluorescence spectra were obtained at 345 nm excitation.

The Sensing Procedure of P_1. Upon testing various conditions, the following procedure was used to study the concentration-dependent changes of P_1 in fluorescence experiments: 10 μL of T_1 solution of a specific concentration were mixed with 10 μL of P_1 solution (50 nM) and the mixture was incubated for 30 min at room temperature. The resulting solution was subsequently mixed in a 13 μL solution

of polymerase-induced replication consisting of 39 mM Tris-HCl (pH 7.5), 27 mM $MgCl_2$, 0.39 mM dithiothreitol (DTT), 1.2 mM dNTPs and 8 U KF^- polymerase, followed by incubation in 37 °C for 60 min. Prior to fluorescence measurements, 42 μL of SG solution (1× conc.), diluted from the stock were injected into the resulting solution. When the time-dependent incubation process was monitored, the fluorescence spectra were collected after a certain period (from 0 to 90 min) at 37 °C, and the relative fluorescence intensity was plotted as the function of incubation time at selected time intervals.

Table S1. Oligonucleotides Used in This Work*

Entry	Sequence(5′-3′)
P_0	GACACCCACCCATCCTCTTAGCGGT(Py) GTCATTTAATTTTGACA-Py
P_1	GACACCCACCCATCCTCTTAGCGGTGTCATTTAATTTTGACA
P_2	CTCCGACACCCACCCATCCTCTTAGCGGTGTCATTTAATTTTGACA
P_3	CACTCCGACACCCACCCATCCTCTTAGCGGTGTCATTTAATTTTGACA
P_4	CTCACTCCGACACCCACCCATCCTCTTAGCGGTGTCATTTAATTTTGACA
P_5	AGGTCTCACTCCGACACCCACCCATCCTCTTAGCGGTGTCATTTAATTTTGACA
T_1	GCTAAGAGGATGGGTGGGTGT
T_{2-1}	GCTAAGAGGATGGGTGGCTGT
T_{2-2}	GCTAAGAGGATGGCTGGGTGT
T_{2-3}	GCTAAGTGGATGGGTGGGTGT
T_{2-4}	GCTTAGAGGATGGGTGGGTGT
T_3	GCTAAGTGGATGGCTGGGTGT

* Py represents the fluorophore pyrene.

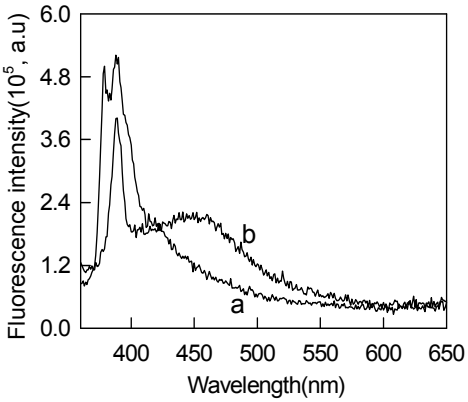

Figure S1. Fluorescence emission spectra of 50 nM P0 (a) and 50 nM P0+ 100 nM T1 (b) in 20 mM Tris-buffer (100 mM NaCl, 5 mM MgCl2) at pH 8.0.

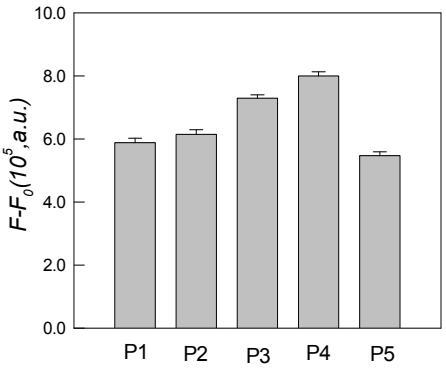

Figure S2. Fluorescence intensity increment of 50 nM target-driven molecular switch (P_1, P_2, P_3, P_4 and P_5, respectively) upon addition of 20 nM T_1 in 20 mM Tris-buffer, 100 mM NaCl, 5 mM $MgCl_2$ at pH 8.0. λ_{ex}=498 nm, λ_{em}=520 nm.

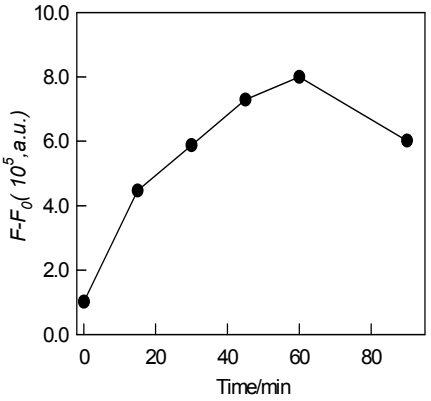

Figure S3. Fluorescence intensity increment of 50 nM P_4 upon addition of 20 nM T_1 as a function of incubation time in 20 mM Tris-buffer, 100 mM NaCl, 5 mM $MgCl_2$ at pH 8.0.

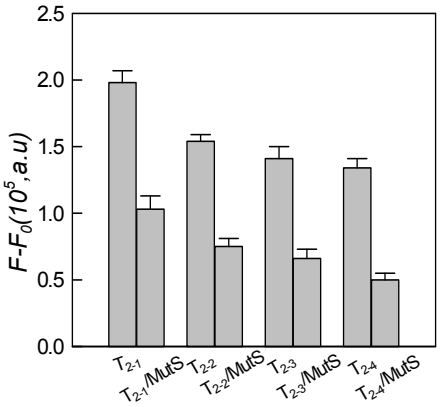

Figure S4. Fluorescence intensity increment of 50 nM P_4 induced by different targets (20 nM) under the same conditions. F and F_0 were the fluorescence intensity observed in the presence and absence of targets, respectively.

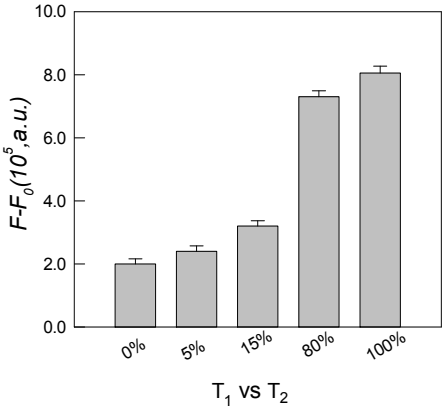

Figure S6. The effect of the T_1 to $T_{2\text{-}1}$ on the detection of SNP. The total concentration of T_1 and $T_{2\text{-}1}$ was 20 nM.[P_4]=50 nM.

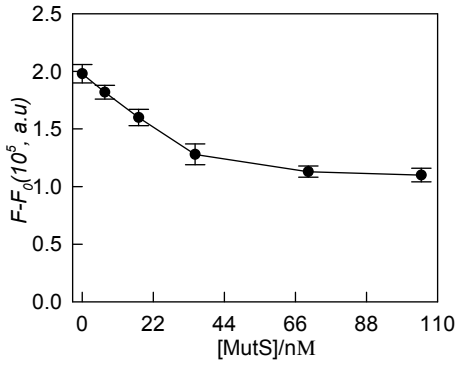

Figure S6. The effect of the concentration of MutS on the detection of $T_{2\text{-}1}$. [P_4]=50 nM, [$T_{2\text{-}1}$]=20 nM. *F* and F_0 were the fluorescence intensity observed in the presence and absence of targets, respectively.