Electronic Supplementary Information

Single-Base Mismatch Discrimination by T7 Exonuclease with Target Cyclic Amplification Detection

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Experimental section

Martials and reagents. T7 exonuclease was purchased from New England Biolabs (Beijing), Ltd. DNA and FAM-labeled probe were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of these DNA and FAM-labeled probe are given in Table S1. Graphene Oxide was purchased from Najing XFNANO materials Tech Co., Ltd. (Najing, China). GeneRuler Ultra Low Range DNA Ladder was obtained from Thermo Fisher Scientific Inc. (Waltham, MA, UAS). 100bp plus DNA ladder and 5 × TBE buffer (225 mM Tris-Boric Acid, 50 mM EDTA, PH 8.0) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All products were of analytical grade and used without further purification. Ultrapure water (18.3MΩ) which was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) was used in the experiments.

Real-Time Fluorescence Analysis. Real-Time Fluorescence Analysis was performed in a 30 μL mixture containing 1 × T7 exonuclease buffer (40 mM Tris-HCl, 20 mM MgCl2, 50 mM NaCl, pH 7.5), 1 U/μL T7 exonuclease, probe 1 (1 μM), wild-type target (100 nM) or mutant-type target (100 nM). The reaction was performed at 25 °C in a C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with a CFX96 in suit detection system. The real-time fluorescence intensity was monitored in 30 s intervals using the FAM/SYBR Green channel. All samples were measured in triplicate. For the Lineweaver-Burk analysis, enzymatic digestion reaction was conducted at different analytes concentration of 10 nM, 20 nM, 40 nM, 80 nM, 100 nM and 200 nM while the concentration of probe 1 is 1 μM. The initial velocity was calculated by

the reported method.[1]

Single-Nucleotide Polymorphism Detection. SNP Detection was performed in a 30 μL mixture containing 1 × T7 exonuclease buffer, 0.26 U/μL T7 exonuclease, 80 nM FAM-labeled probe and various concentrations of target at 25 °C for 2 h, then 2.5 μL 200 μg/mL prepared single-layer graphene oxide nanosheets solution and ultrapure water were added into the reaction solution with a final volume of 100 μL. The reaction was then incubated at room temperature for 5 min before fluorescence assay. The fluorescence spectra were collected at room temperature using a quartz cuvette on an F-7000 Fluorescence Spectrophotometer (Hitachi, Japan). The excitation wavelength was 494 nm and the emission wavelength was from 509 nm to 600 nm.

Gel electrophoresis analysis. Gel electrophoresis analysis was performed using 4% (w/w) agarose gels containing 0.5 μg/mL ethidium bromide (EB) and 0.5 μg/mL Goldview in 0.5 × TBE buffer. The electrophoresis was then performed at a constant potential of 101 V for 2 h with a load of 10 μL of sample in each lane at room temperature. After electrophoresis, the gel was imaged via a Tocan 240 gel imaging system (Shanghai Tocan Biotechnology, Shanghai, China).

Human Genome DNA Sample Analysis. Targets for human genome sample analysis were prepared by PCR.[2] Briefly, the beta-thalassemia gene was amplified using 25ng of genomic DNA in a 50 μL reaction volume containing 1 × Taq buffer, 2 mM $MgCl_2$, 100 nM of each primer, 200 μM dNTP and 1U Taq DNA polymerase. After an initial hot start of 95 °C for 5 min, 40 cycles amplification of 95 °C for 10 s, 58 °C for 20 s and 72 °C for 10 s with a final extension time of 7 min were performed, then added

another 100 nM forward primer, and run 1 cycle under the same conditions to get the 373 nt ssDNA targets. All PCR products were directly used in the next experiments without any other treatment.

Table S1. Sequences of Oligonucleotides and probes used in this work.

Name	Sequences（5′-3′）
Probe 1	FAM-ACCTT(Dabcyl)AACCCAG
Probe 2	FAM-ACCTTAACCCAG
Wild-type target	TCTAAAGAATAACAGTGATAATTTCTGGGTTAAGG CAATAG
Mutant-type target	TCTAAAGAATAACAGTGATAATTTCTGGGTTAAGG TAATAG
Forward primer	AATGTATCATGCCTCTTTGCACC
Reverse primer	TGGGCCAGGGCATTAGCC

Mutant-type target: Part sequence of Beta-Thalassemia, the mutant site (IVSⅡ-654) is marked in red.

Table S2. Comparison of various methods for SNP detection.

Analytical method	Detection limit	Reference
GO-based sensor without amplification	1 nM	3
GO-based sensor without amplification	40 pM	4
GO-based sensor with rolling circle amplification	10 pM	5
GO-based sensor with Exo ⅢIamplification	5 pM	6
GO-based sensor without amplification	650 pM	7
GO-based sensor without amplification	2 nM	8
GO-based sensor without amplification	3 nM	9
GO-based sensor with T7 exonuclease amplification	4 pM	This work

Fig. S1. SNP discrimination by T7 exonuclease with target cyclic amplification.

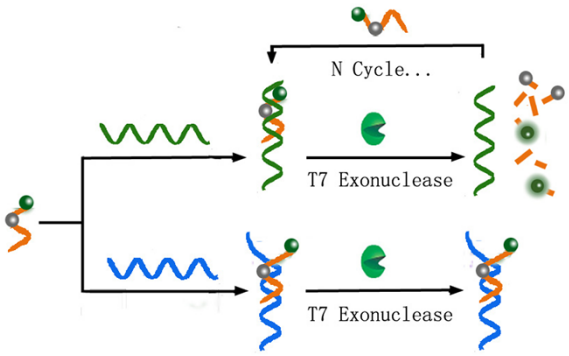


Fig. S2. AFM image of GO on the mica substrate.

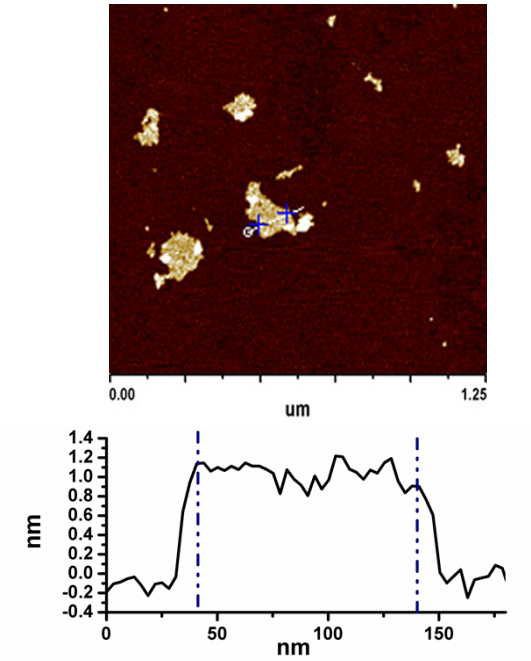


Fig. S3. Optimizing the amount of T7 exonuclease. F and F_0 represent the fluorescence signal in the present of DNA 2 and DNA 1, respectively. Error bars are standard deviation of three repetitive experiments.

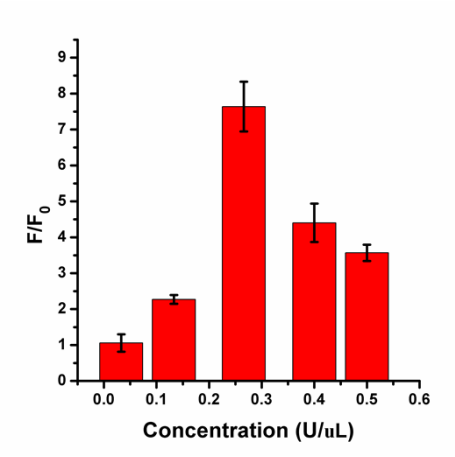


Fig. S4. Fluorescence spectrum of varying percentage (0, 0.1%, 0.5%, 1%, 2%, 4%, 10%, 20%, 40%, 50%, 100%, from bottom to top) of mutant-type target in mixed DNA samples. Mixed DNA samples were prepared by mixing mutant type target and wild type target at different ratios, and total concentration of the wild and mutant type analytes is 20 nM.

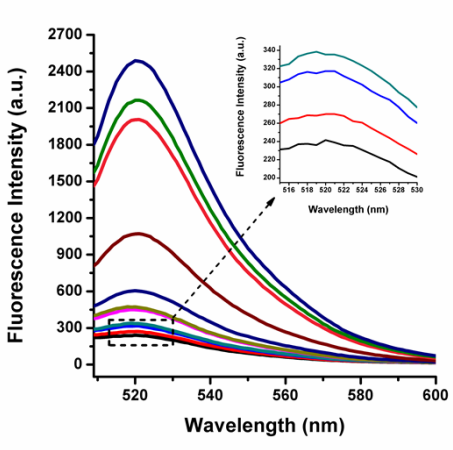


Fig. S5. Agarose gel electrophoresis image of PCR products. Lane 1-2: wild-type samples; Lane 3-6: mutant-type samples; Lane M is DNA size marker. Dark bands were observed for the PCR amplicons near the position of DNA marker with a length 500 base-pairs. This is consistent with the length of target amplicons with predicted 373 base-pairs.

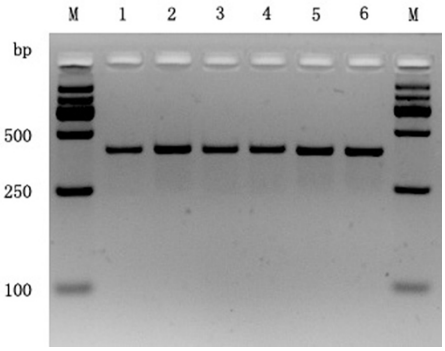
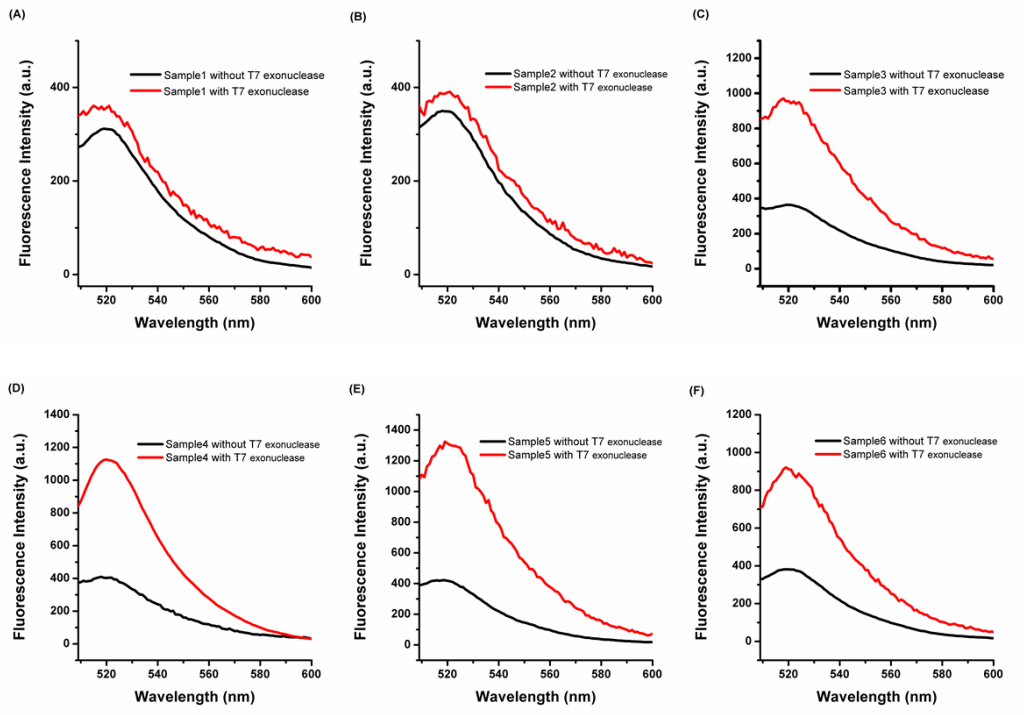


Fig. S6. Fluorescence Spectrum of the human genomic samples. Probe (80nM) reacted with the PCR products of wild type samples (1 and 2) and mutant type samples (3, 4, 5 and 6).



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