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Assistant DNA recycling with nicking endonuclease and molecular beacon for signal amplification using a target-complementary arched structure

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Experimental

Materials and reagents: Nicking endonuclease Nt.BbvCI and NEB buffer 4 were purchased from New England Biolabs (Ipswich, MA, USA). Tris-(hydroxymethyl) aminomethane (Tris) was purchased from Sigma-Aldrich Inc. Phosphate buffer saline (PBS) was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. DNA hybridization buffer contained 10 mM PBS (pH 7.4), 137 mM NaCl, and 2.5 mM Mg^{2+}. Human serum samples were kindly provided by the Jiangsu Cancer Hospital (Nanjing, China). A mixture containing equal volumes of human serum sample and 1 mM ethylenediaminetetraacetic acid (EDTA) in 10 mM Tris-HCl was used for recovery testing. DNA oligonucleotides were synthesized and purified by Takara Biotechnology Co., LTD. (Dalian, China), and stored in Tris-HCl (10 mM, pH 8.0) containing 1 mM EDTA. The sequences of these oligonucleotides are as follows:

Probe 1: 5′-FAM-CATCTCTTCTGAGTGGATCCAGTACTGACGCCGTATAGTGAGCA-3′;

Assistant DNA (A-DNA): 5′-TGCTCACTATGCTGAGGAGAAGAGATG-BHQ-3′;

Molecular beacon (MB): 5′-BHQ-TCGATTACTTCT**CCTCAGC**ATAGTAATCGA-FAM-3′;

Target DNA: 5′-CTCACTATACGGCGTCAGTACTGGATCCACTCAGAAGAGA-3′;

Single-base mismatched DNA:

5′-CTCACTATACGGCGTCAGTAC**A**GGATCCACTCAGAAGAGA-3′;

Two-base mismatched DNA:

5′-CTCACTATACGGC**C**TCAGTAC**A**GGATCCACTCAGAAGAGA-3′;

Three-base mismatched DNA:

5′-CTCACTATACGGC**C**TCAGTAC**A**GGATCC**T**CTCAGAAGAGA-3′.

Here FAM and BHQ are fluorescent dye, carboxyfluorescein, and its quencher, respectively. Probe 1 has 2×10 bases in the stem, which all hybridized with the two ends of A-DNA (red and blue parts) to form the arched structure. After hybridization of P1 with target DNA, the released A-DNA hybridized with MB by 17 underlined bases, in which the bold part with 7 bases was the recognition site of NEase for signal amplification.

Fluorescent assay: In a typical DNA assay, the hybridization of P_1 (1 μM) with assistant DNA (1 μM) was first performed in the reaction buffer for 1 h at room temperature to form the arched structure. After mixing 20 μL of the arched structure (1 μM) with 20 μL varying concentrations of target DNA, 20 μL MB (10 μM), 100 μL NEB buffer 4, and 5 U Nt.BbvCI were added simultaneously at 37 °C for 40 min to perform assistant DNA recycling reaction, and then kept at 80 °C for 10 min to deactivate the nicking enzyme. The result solution was diluted to 0.4 mL with PBS for fluorescence detection. Fluorescence measurements were conducted on a F900 fluorescence spectrometer (Edinburgh Instruments Ltd., UK). The fluorescence spectra were

obtained by scanning from 500 to 620 nm with a step of 1 nm at an excited wavelength of 496 nm.

Gel electrophoresis: A 20 % polyacrylamide gel electrophoresis analysis was carried out in 1×Tris-Borate-EDTA (pH 8.3) at 110 V constant voltage for about 2 h. After ethidium bromide staining, gels were scanned using a Molecular Imager Gel Doc XR (BIO-RAD, USA).

DNA detection without NEase amplification

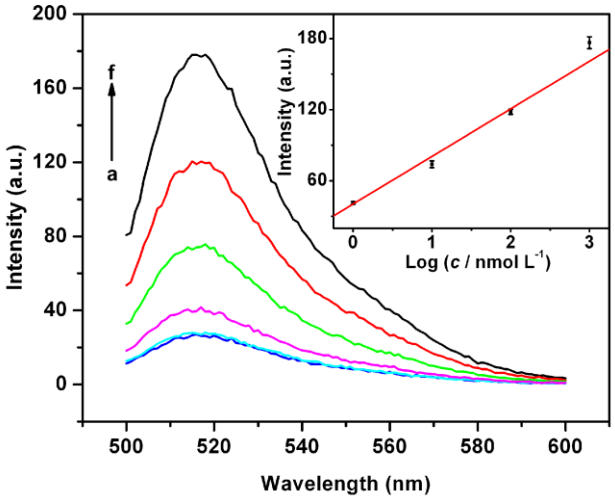

Fig. S1 Fluorescence spectra for blank (a) and target at 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} and 10^{-6} mol L^{-1} (from b to f) without presence of nicking endonuclease. Inset: plot of fluorescence intensity *vs* logarithm of target concentration.

Fluorescence intensity for mismatched DNA at different concentrations

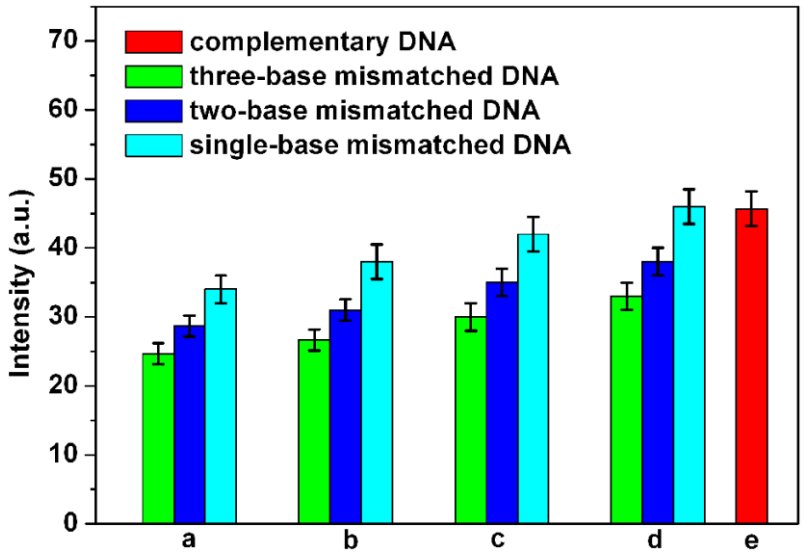

Fig. S2 Histograms of fluorescence intensity for mismatched DNA at 10^{-9} (a), 10^{-8} (b) 10^{-7} (c), 10^{-6} mol L^{-1} (d) and complementary DNA at 10^{-12} mol L^{-1} (e).