Electronic Supplementary Information

dsDNA-specific fluorescent copper nanoparticles as a "green" nano-dye for polymerization-mediated biochemical analysis

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Chemicals and Materials. All dNTPs and oligonucleotides of HPLC purity were purchased from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences of oligonucleotides used for the polymerase detection are listed in Table S1, the sequences of oligonucleotides used for mercury ion detection are listed in Table S2, and the sequences of oligonucleotides used for the nucleic acid amplified detection are listed in Table S3. All oligonucleotides were dissolved in sterile deionized water, ten micromolar of each oligonucleotide was prepared as the stock solution. Klenow Fragment of *E. coli* DNA Polymerase I (KF), *E. coli* DNA ligase, and bovine serum albumin (BSA) were obtained from New England Biolabs (NEB, UK). Klenow Fragment exo$^-$ of *E. coli* DNA Polymerase I (KF$^-$), RevertAid M-MuLV reverse transcriptase, and S1 nuclease were purchased from Thermo Fisher Scientific Inc. (America). 3-(N-morpholino) propanesulfonic acid (MOPS), sodium ascorbate, copper sulfate, mercuric nitrate and other salt reagents were commercially obtained from Dingguo Biotechnology Co., Ltd. (Beijing, China). Chloroquine, mannitol and glucose were purchased from Aladdin Industrial Inc (Shanghai, China). All salts were at least analytical grade and used without further

treatment. Several buffers were used in the present assays: 1×NEBuffer 2 (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9@25 °C) was used for KF dilution and primer-extension reaction in the polymerase detection, 1 mg mL^{-1} BSA was mixed into the 1×NEBuffer 2 to reduce the absorption of KF onto plastic wall in the dilution and reaction process; 1×Reaction buffer (50 mM Tris-HCl, 50 mM $MgCl_2$, 1 mM DTT, pH 8.0 @25 °C) was used for KF^- dilution and primer-extension in the mercury ion detection; 1×PCR buffer (20 mM Tris-HCl, 10 mM KCl, 16 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% TritonX-100, pH 8.8@25 °C) was used for PCR reaction in nucleic acid detection; MOPS buffer (10 mM MOPS, 150 mM NaCl, pH 7.8@25 °C) was used for the formation of fluorescent CuNPs. Deionized water was prepared through the Nanopure Infinity™ ultrapure water system (Barnstead/Thermolyne Corp.).

Characterization. Fluorescence measurements were carried out on an F-7000 fluorescence spectrophotometer (Hitachi, Japan) equipped with an aqueous thermostat (Amersham) which is accurate to 0.1 °C. Excitation wavelength was set at 340 nm, and fluorescence emission spectra were obtained by collecting emission intensity from 500 to 660 nm with a 0.2 cm × 1 cm quartz cuvette containing the detected solution. All measurements were carried out at room temperature (25 °C) unless stated otherwise.

Transmission electron microscopy (TEM) measurements were performed on a JEM-3010 transmission electron microscope (JEOL, Japan) operated at 300 kV. TEM samples were prepared by spin coating 10 μL of CuNPs onto carbon-coated copper grid substrates, which were then baked in an oven at 60℃.

3% agarose gel electrophoresis was used to characterize the polymerization production. First, 8 μL DNA sample was mixed with 2 μL of 6×loading buffer and 2 μL of 100×SYBR Gold. Then, 10 μL mixture was injected into the gel, and the gel was run at 100 V for 2 h in 1×TAE buffer (40 mM Tris AcOH, 2.0 mM Na_2EDTA, pH 8.5@25 °C). Finally, the gel was illuminated with UV light and finally photographed by the Tanon-2500R gel imaging system (Shanghai, China).

During the gel staining by CuNPs, first, 1.5% agarose gel was prepared by using MOPS buffer (10 mM MOPS, 150 mM NaCl, 5 mM Vc, pH 7.8@25 °C), and 8 μL DNA sample was mixed with 2 μL of 6×loading buffer. Then, 10 μL mixture was injected into the gel, and the gel was run at 20 V for 10 min in MOPS buffer. Finally, the gel was soaked in MOPS buffer containing 2 mM copper sulfate for 10 min, and the gel was quickly photographed in Tanon-

2500R gel imaging system.

Polymerase Detection. KF polymerase was first diluted to different concentrations using the 1×NEBuffer 2. 40 μL reaction system containing 0.8 μM template, 0.8 μM primer, 250 μM dNTPs (each) and KF of various concentrations was used to carry out the primer-extension reaction. After incubation for 2 h at 37 °C in PCR tube, the primer-extension reaction was stoped by heating at 75 °C for 20 min. Then, 150 μL MOPS buffer was added into the above solution, 10 μL sodium ascorbate of 100 mM and 2.4 μL copper sulfate of 10 mM were mixted with the polymerization products, triggering the formation reaction of the fluorescent copper nano-dye on the dsDNA scaffolds. Finally, after another incubation at ambient temperature (25°C) for 5 min, 200 μL of the resulted solution was added into the quartz cuvette for the fluorescence measurement. The inhibition experiments were the same as the above procedure, except for 30 min preferential incubation of KF with inhibitor before its addition into the reaction system.

To achieve a better sensing performance for polymerase assay, a series of conditions, including the primer length, the ratio of template to primer, and the polymerization time, were optimized through the manner of signal-to-background ratio (SBR) (SBR = F/F_0), where F was the fluorescence emission intensity of the copper nano-dye at 570 nm in the presence of KF, and F_0 was that in the absence of the KF. As shown in Fig. S2, those results illustrated that the primer length of 12-mer, the template to primer ratio of 1:1, and the polymerization time of 2 h could result in maximum SBR for the polymerase sensing system, which were used as the optional conditions for the polymerase detection in the further experiments.

Mercury Ion Detection. First, mercury ion was mixed with 5 μL of template (10 μM) and 5 μL primer 12-2T (10 μM), and the mixture was incubated at room temperature (25 °C) for 20 min to ensure that template and primer were hybridized to each other completely with the binding of mercury ion. Then, mercury ion-assistant primer-extension reaction was carried out in a 20 μL solution containing the above hybridization mixture, 1×Reaction buffer, 250 μM dNTPs (each), and 2 units KF^- polymerase. After incubation for 90 min at 37 °C, the polymerization was terminated by heating at 75 °C for 10 min. Then, 75 μL MOPS buffer was added into the above solution, 3 μL sodium ascorbate of 100 mM and 3 μL copper sulfate of 10 mM were mixted with the polymerization products, triggering the formation reaction of the fluorescent copper nano-dye on the dsDNA. Finally, after incubation at ambient temperature (25°C) for 5 min, the fluorescence

spectra of the resulted solutions were measured at room temperature (25 °C).

Nucleic Acid Detection. Nucleic acid detection by fluorescent copper nano-dye was carried out by taking advantage of the amplification capability of polymerase chain reaction (PCR). In detail, the PCR reaction was performed in a solution of 20 μL volume. The reaction mixture contained 2.5 μM forward primer, 2.5 μM reverse primer, 2.5 units of Pfu polymerase and the target DNA of different concentrations. Cycling parameters for PCR in this work were set as follows: initial denaturation at 94 °C for 1 min, followed by 30 cycles of 94 °C for 30 s, 62.2 °C for 30 s, 72 °C for 30 s, and a final extension step at 72 °C for 10 min. After PCR, the amplified PCR products were quantified by fluorescent copper nano-dye: PCR products were first mixed with 80 μL MOPS buffer, 5 μL sodium ascorbate of 100 mM and 3 μL copper sulfate of 10 mM were then introduced into the above mixture for triggering the formation reaction of the fluorescent copper nano-dye, incubation for another 5 min, the fluorescence spectra of the mixtures were recorded at room temperature (25 °C).

Since the cycle number and the annealing temperature are two important parameters for PCR performance, they were optimized to make the detection system exhibit the best signal-to-background ratio (SBR) (SBR = F/F_0), where F was the fluorescence emission intensity of the copper nano-dye at 570 nm in the presence of the target DNA, and F_0 was that in the absence of the target DNA. As shown in Fig. S7, the detection system exhibited the best SBR with the cycle number of 30 and the annealing temperature of 62.2 °C, which were chosen as the optimal parameters for the further DNA detection experiments.

Table S1. Oligonucleotides used for the polymerase assay in this study.

type	sequence (from 5' to 3')
template	TACTCATACGCTCATACGTTCATCACGACTACTTGTACTTCCC GTC
c-template	GACGGGAAGTACAAGTAGTCGTGATGAACGTATGAGCGTAT GAGTA
primer-6	GACGGG
primer-8	GACGGGAA
primer-10	GACGGGAAGT
primer-12	GACGGGAAGTAC
primer-14	GACGGGAAGTACAA

Table S2. Oligonucleotides used for the mercury ion assay in this study.

type	sequence (from 5' to 3')
template	TACTCATACGCTCATACGTTCATCACGACTACTTGTACTTCCC GTC
primer-12-2T	GACGGGAAGTACTT

Table S3. Oligonucleotides used for the nucleic acid assay in this study.

Type	Sequence (from 5' to 3')
target DNA	CACAGTGAGTCTCCCCTTTCCTTTCCTTGTATCTTCTTCCTTTC TTTCTTTCGACTAAGCACCATCTCTTCTCCGAGTCGGTGACAC T
control DNA	TGACTACGCACCATAGTATGGTAGTGAAGTATCTACTTCCTA TCTTACTATTACACAAGCACCATGTCTGACACTACTATGCAC AATC
forward primer	AGTGTCACCGACTCG
reverse primer	CACAGTGAGTCTCCC

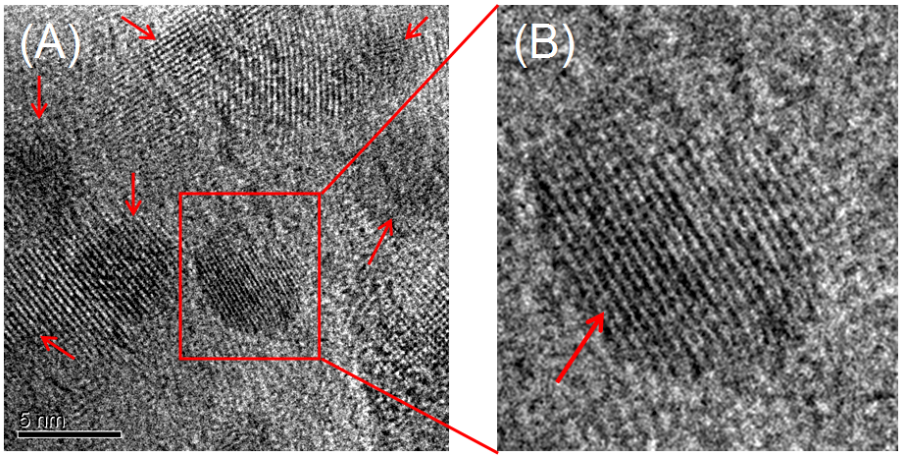

Fig. S1 TEM images of CuNPs templated by a dsDNA. The right (B) is the enlarged picture of the square in the left (A). The arrows mark the positions of CuNPs.

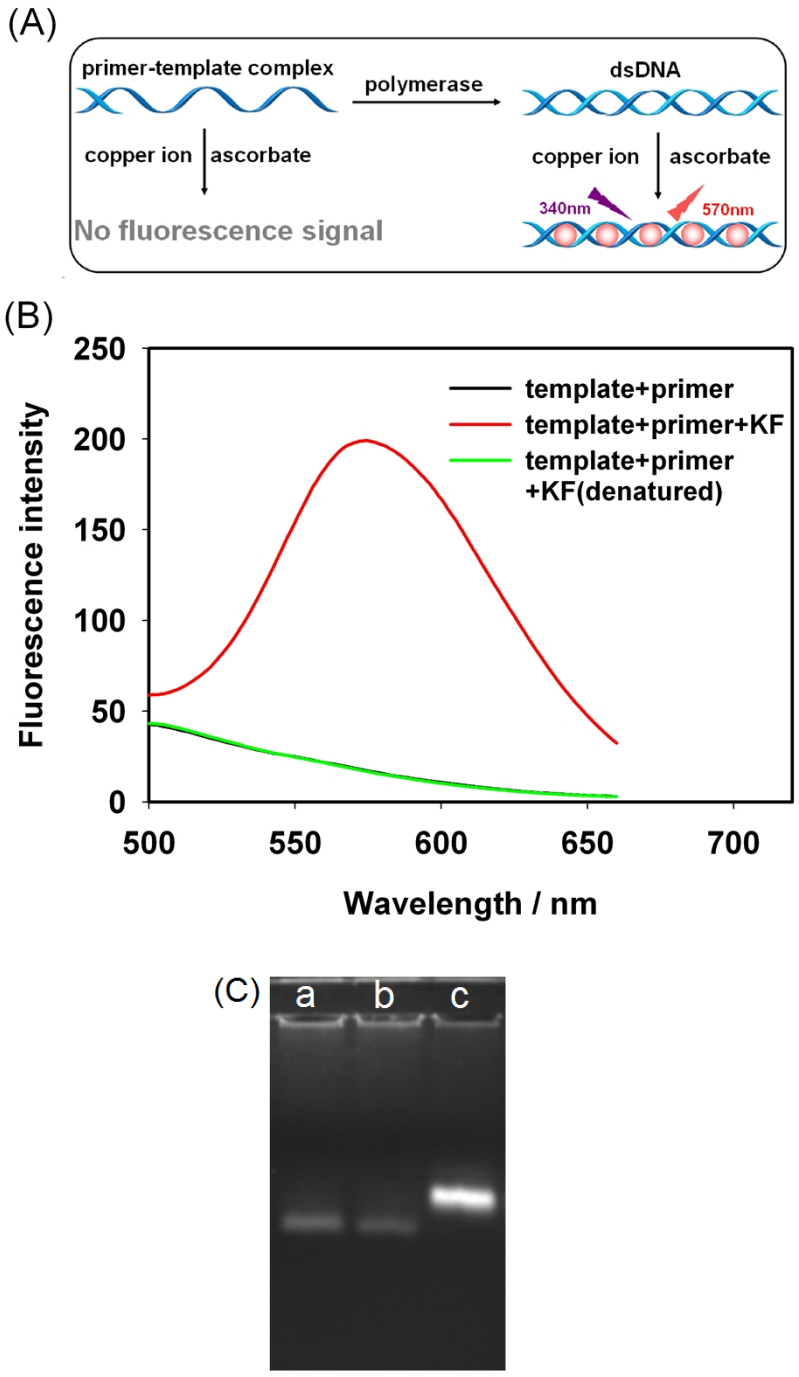

Fig. S2 (A) Schematic representation of the proposed strategy for polymerase detection. (B) Feasibility verification by CuNPs' fluorescence. (C) Verification of primer-extension reaction by gel electrophoresis: a, template + primer; b, template + primer + KF (denatured); c, template + primer + KF. Only in the presence of active polymerase, primer-extension reaction can be achieved.

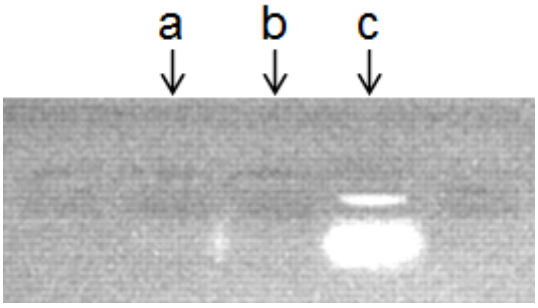

Fig. S3 Verification of the potential of the copper nano-dye for gel staining. a, single strand DNA (template); b, single strand DNA (c-template); c, double strand DNA (template+ c-template). The copper nano-dye is specific for double strand DNA.

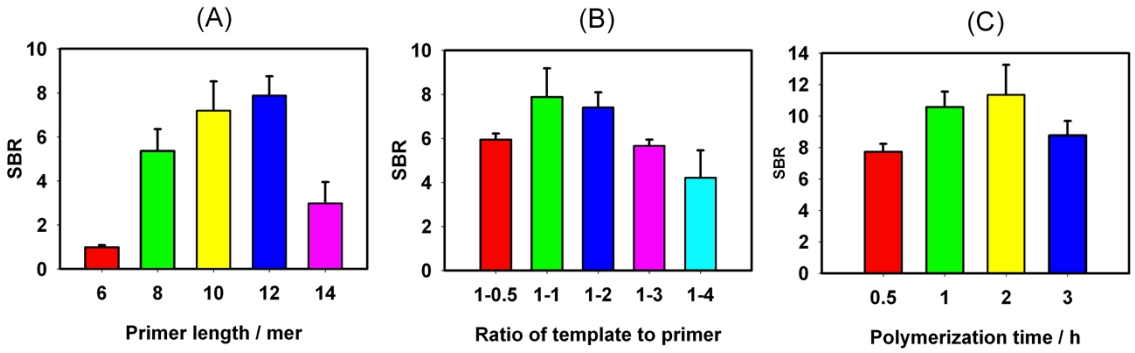

Fig. S4 Effect of the primer length (A), ratio of template to primer (B), polymerization reaction time (C) on the signal-to-background ratio (SBR) (SBR = F/F_0), where F was the fluorescence emission intensity of the copper nano-dye at 570 nm in the presence of 5×10^{-3} units μL^{-1} KF polymerase, and F_0 was that in the absence of the KF polymerase. Those results illustrated that the primer length of 12-mer, the template to primer ratio of 1:1, and the polymerization time of 2 h could result in maximum SBR for the polymerase sensing system, which were used as the optional conditions for the polymerase detection in the further experiments.

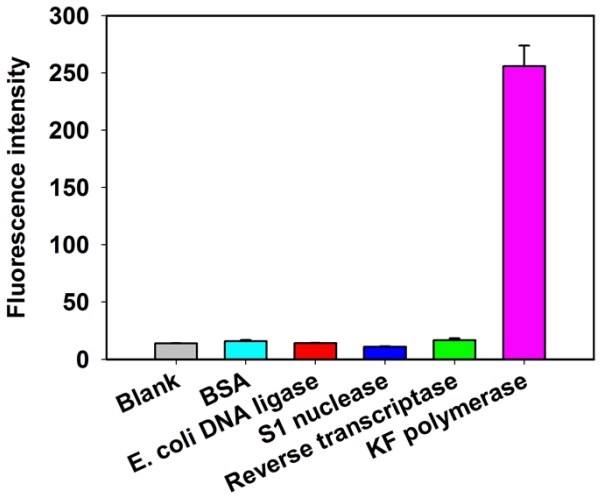

Fig. S5 Selectivity of the assay system for polymerase. The concentration of KF polymerase was 5×10^{-3} units μL^{-1}, the concentration of each of the other enzymes (*E. coli* DNA ligase, S1 nuclease and reverse transcriptase) was 5×10^{-2} units μL^{-1}; the bovine serum albumin (BSA) was 1mg mL^{-1}. The error bars represent the standard deviation of three independent measurements.

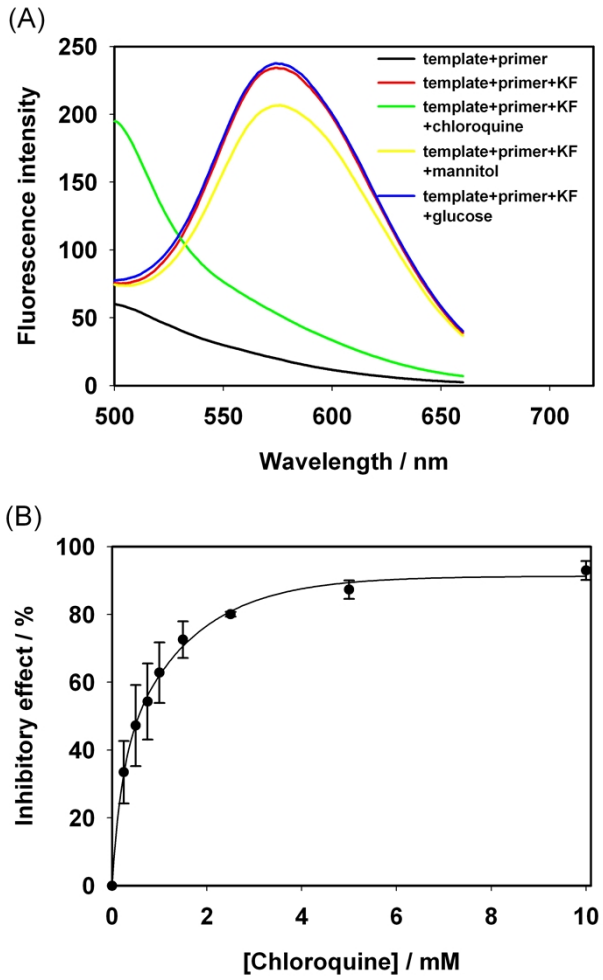

Fig. S6 Demonstration of the potential capability of the strategy for polymerase inhibitors screening. (A) Fluorescence spectra of the polymerase assay system in the presence of different inhibitors. [KF] = 5×10^{-3} units μL^{-1}, [chloroquine] = [mannitol] = [glucose] = 1.5 mM. (B) Relationship between the inhibitory effect and the concentration of the inhibitor chloroquine.

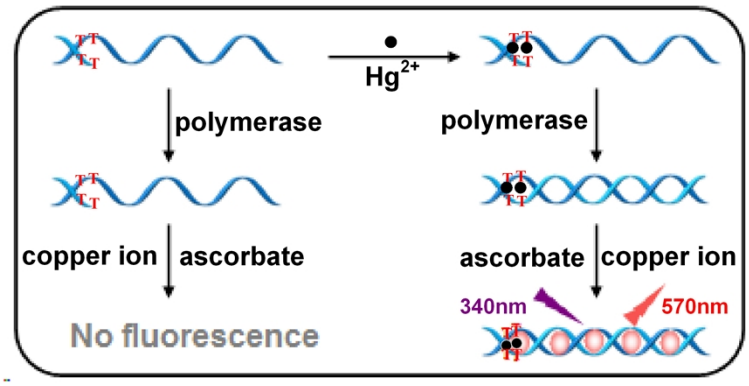

Fig. S7 Schematic representation of the proposed strategy for mercury ion detection based on mercury-mediated complementary primer-template complex and subsequent primer-extension reaction.

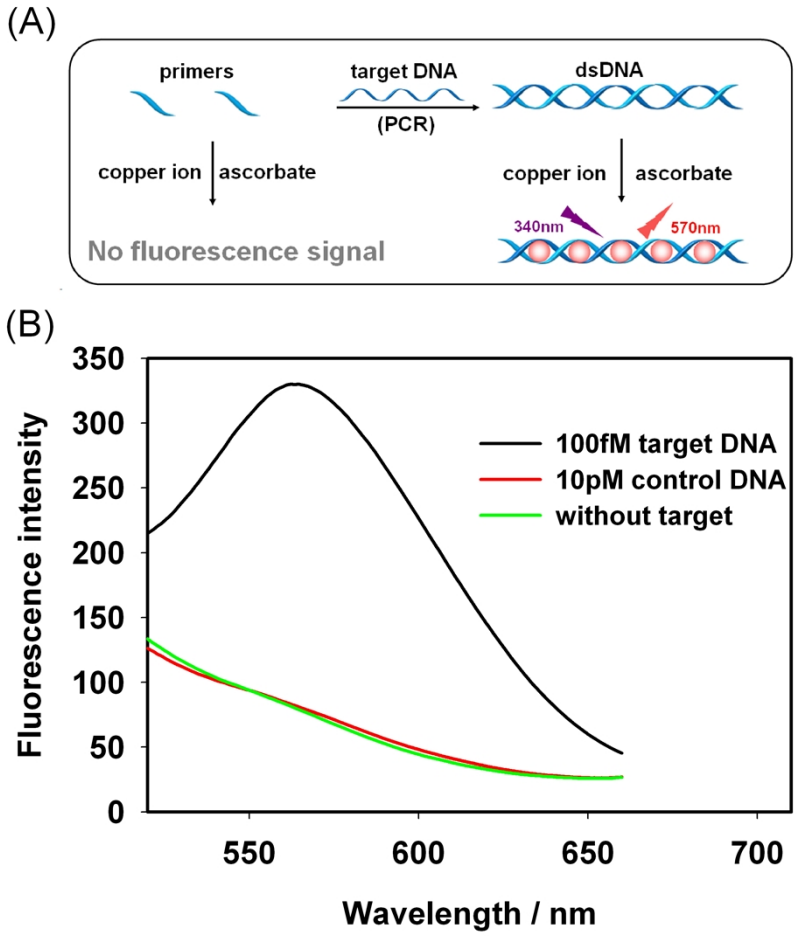

Fig. S8 (A) Schematic representation of the proposed strategy for DNA amplified detection by combining PCR technology. (B) Feasibility verification.

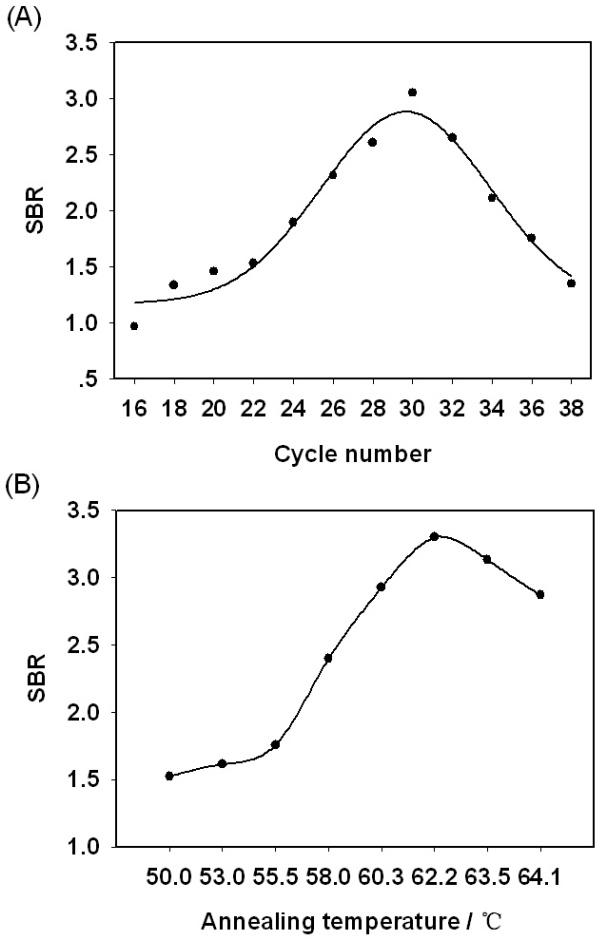

Fig. S9 Effects of the cycle number (A) and annealing temperature (B) on the signal-to-background ratio (SBR) (SBR = F/F_0), where F was the fluorescence emission intensity of the copper nano-dye at 570 nm in the presence of 0.1 fM target DNA, and F_0 was that in the absence of the target DNA. Those results illustrated that the detection system exhibited the best SBR with the cycle number of 30 and the annealing temperature of 62.2 °C, which were chosen as the optimal parameters for the further DNA amplified detection experiments.