Supporting Information

Tandem Blocking of PCR Extension to Form Single-stranded Overhang for Facile, Visual, and Ultrasensitive Gene Detection

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Abstract

In order to detect a predetermined gene in field test, facile, visual, and ultrasensitive approach without the need of special and expensive machines is required. In this study, a gene in Ebola virus was targeted as an example for diagnosis. The key strategy is to incorporate molecular blockers (azobenzene-bearing moieties or thymine dimers) in tandem in one of the PCR primers and stop the polymerase extension to form single-stranded overhang. The PCR product was added to the dispersion of gold nanoparticles which were labelled with a probe oligonucleotide. When the Ebola virus-specific gene existed in the specimen, the oligonucleotide on the gold particles formed a double-helix with the single-stranded overhang, and thus the dispersion remained red in color. In the absence of the gene, however, the dispersion rapidly turned to blue because of nanoparticles aggregation. The difference was explicit even when the initial specimen involved only 1 copy of the gene. Accordingly, “whether the patient is infected by the virus or not” can be easily and visually judged by our naked eyes.

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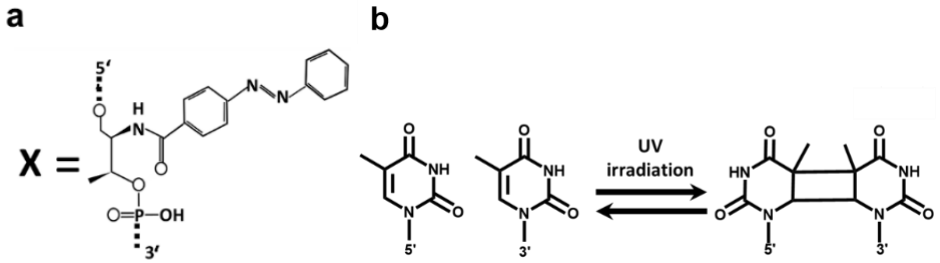

Fig. S1 Structures of the molecular blockers used in this assay.

a) X group bearing an azobenzene;[1] b) thymine dimer formed by UV irradiation (Only cyclobutane dimer is presented).[2]

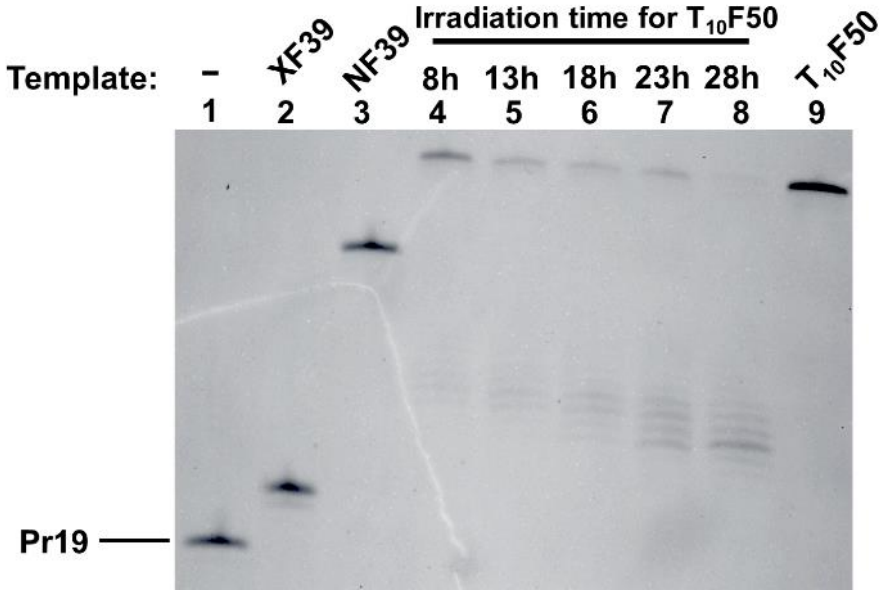

Fig. S2 Effect of photo-irradiation time on the formation of thymine dimers, evaluated by their blocking activity on primer extension (the products were analyzed by PAGE electrophoresis).

With a handheld UV lamp (0.4 W/m^2), $T_{10}F50$ involving 10 consecutive thymines was irradiated for various period of time, and used as the template for extension of Pr19 primer. Lane 1, Pr19 only; lane 2, Primer extension on template XF39 involving two X's; lane 3, Primer extension on NF39 (a non-modified template without two X's or thymines). Lane 4~8: Primer extension at various irradiation time. Lane 9: Primer extension on $T_{10}F50$ without irradiation.

Note that the amount of run-through product (in the top of the gel) gradually decreased with increasing time of photo-irradiation, and the shorter products concurrently became dominant. With 28 h irradiation, the run-through product was hardly perceived (lane 8).

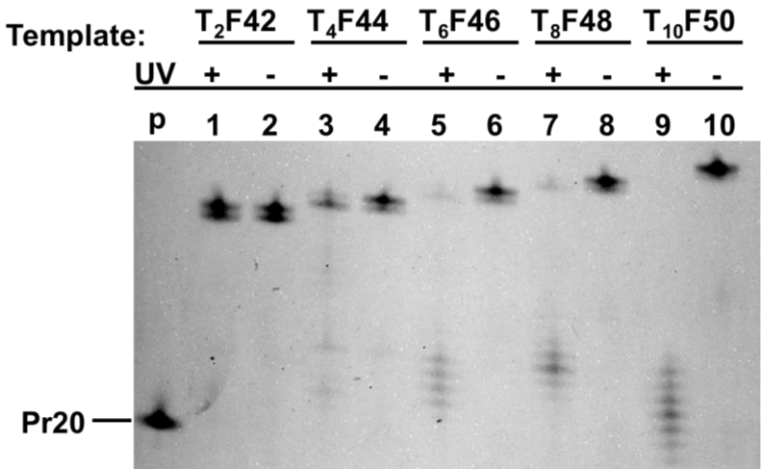

Fig. S3 Effect of the length of continuous thymines in primer precursors on the formation of thymine dimers, evaluated by their blocking activity on primer extension (the products were analyzed by PAGE electrophoresis). The precursors involving different number of continuous thymines in Table S1 were irradiated for 28 h with a handheld UV lamp (0.4 W/m^2), and used as template for extension of Pr19 primer. Lane P, Pr20 only; lane 1, 3, 5, 7, 9 were the results after photo-irradiation for T_2F42, T_4F44, T_6F46, T_8F48, and T_{10}F50, respectively. Lanes 2, 4, 6, 8, and 10 were controls without photo-irradiation of T_2F42, T_4F44, T_6F46, T_8F48, and T_{10}F50, respectively.

The blocking activity was almost zero for T_2F42 and T_4F46 after a long time of the photo-irradiation. But the activity monotonously increased with number of thymines. When T_{10}F50 with 10 thymines was used, almost complete blocking was accomplished.

Tab. S1 Oligonucleotides used for primer extension experiments in Fig. S3

ODNs	Sequence (5'→3')	Length /nt
Pr20	CAATTTGGACGACGACGATG	20
T_2F42	CCATGTCTAATGCTGATGAT$_{(2)}$ACATCGTCGTCGTCCAAATTG	42
T_4F44	CCATGTCTAATGCTGATGAT$_{(4)}$ACATCGTCGTCGTCCAAATTG	44
T_6F46	CCATGTCTAATGCTGATGAT$_{(6)}$ACATCGTCGTCGTCCAAATTG	46
T_8F48	CCATGTCTAATGCTGATGAT$_{(8)}$ACATCGTCGTCGTCCAAATTG	48
T_{10}F50	CCATGTCTAATGCTGATGAT$_{(10)}$ACATCGTCGTCGTCCAAATTG	50

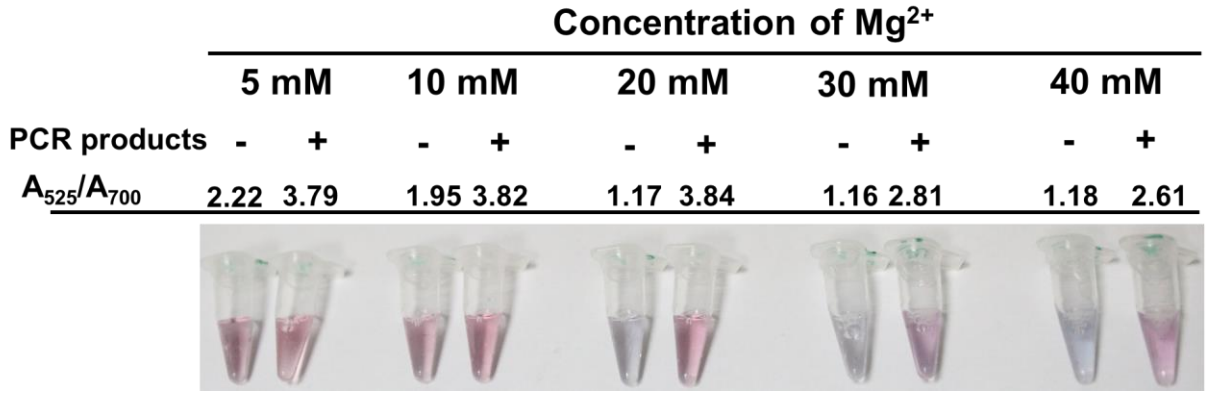

Fig. S4 Effect of the concentration of Mg^{2+} on AuNps dispersion-induced visual detection of the product of indenting PCR. For each concentration of Mg^{2+}, the right tube was for the product of indenting PCR starting from $5x10^3$ gene copies, and the left one was the control starting from the specimen containing no gene.

When the concentration of Mg^{2+} is 5 mM or 10 mM, both the positive sample and the negative sample were red. The differentiation between them was possible only by A_{525}/A_{700} ratios. When $[Mg^{2+}]$ > 20 mM, however, the difference between them could be clearly detected by our naked eyes. For the present detection, 20 mM Mg^{2+} has been chosen, because (1) visual detection of target gene was successfully achieved and (2) the AuNps dispersions were satisfactorily stable (note that A_{525}/A_{700} is smaller at higher $[Mg^{2+}]$).[3]

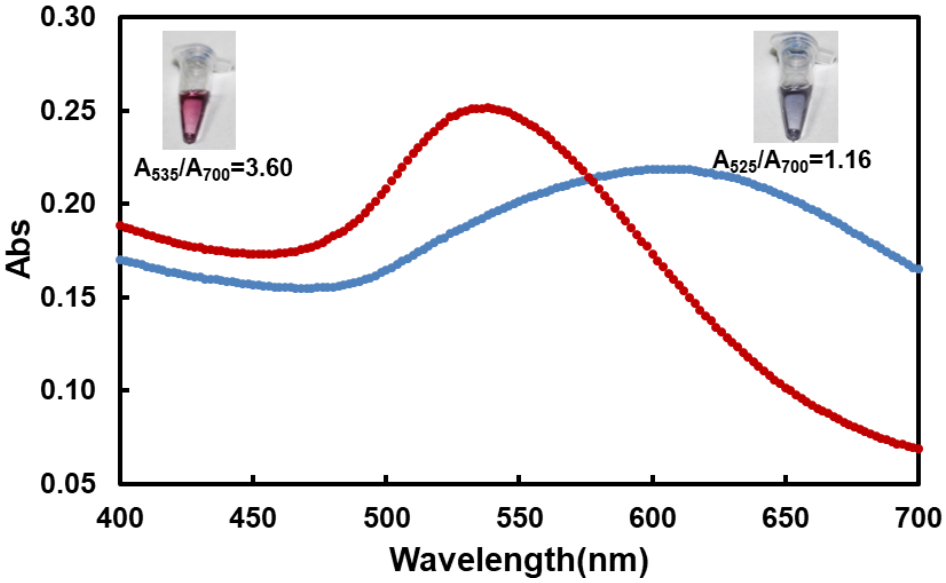

Fig. S5 Visual and spectral detection of the products of indenting PCR, using thymine dimers as molecular blockers, formed by photo-irradiation of T_{10}F50 (red line). The product was added without purification to colloidal AuNps. For the purpose of comparison, the results with the product of conventional PCR using non-irradiated primers (bearing no single-stranded overhang) were shown by blue line. The inserts were the corresponding pictures.

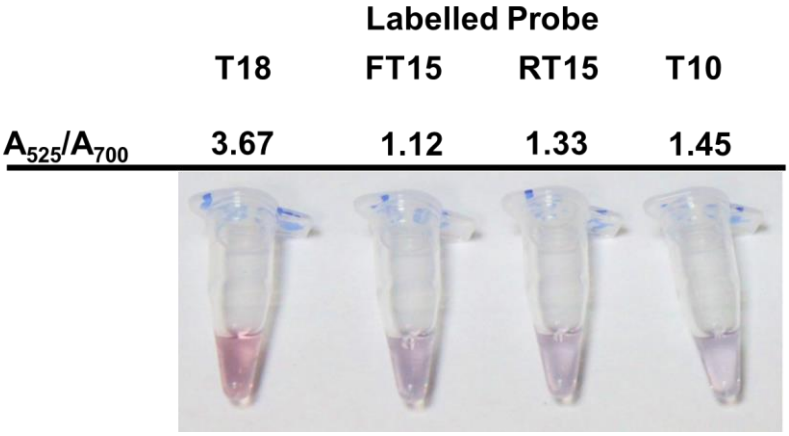

Fig. S6 The specificity of AuNps sensors toward the PCR products was evaluated using the fully complementary (T18), totally mismatched (FT15), and five-base mismatched (RT15) DNA sequences and partially complementary (T10) sequences. The PCR products were amplified by primer pair XF39/R24 under the same condition as products in the Fig. 2. The totally mismatched FT15 was used as control.

As shown in the Fig. S6, the solution kept red only when probe T18 were labeled on the AuNPs; the detection was negative when AuNPs were labelled with mismatched probes (FT15 or RT15) or partially complementary probe (T10). The solution turned into blue since the PCR products couldn't be captured. This indicates the specific detection of PCR products using labelled AuNps, which may be used for multiplex PCR by using several kinds of AuNPs.

Tab. S2 Oligonucleotides used for primer extension experiments in Fig. S6

ODNs	Sequence (5'→3')	Length/nt
XF39	CCATGTCTAATGCTGATxGxACATCGTCGTCGTCCAAATTG	39
R24	GTTGACCCGTATGATGATGAGAGT	24
T18	CATCAGCATTAGACATGG-SH	18
FT15	GTAGTTACAGAGACG-SH	15
RT15	TGGTTTTAGACATGG-SH	15
T10	CATCAGCATT-SH	10

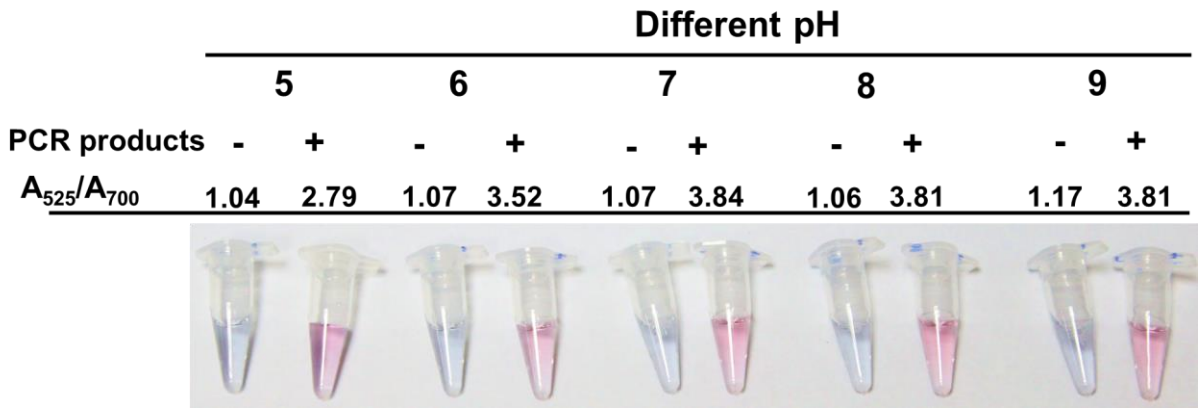

Fig. S7. The Effect of pH on the visual detection. For each pH, the right tube was for the product of indenting PCR starting from $5x10^3$ gene copies, and the left one was the control starting from the specimen containing no gene. For each reaction the final $[Mg^{2+}]$ is 20 mM. The pH value was measured using a standard pH test paper.

As shown in the Fig. S7, in the pH value from 5-9, all the positive samples are red and all the negative samples are blue. The difference between them could be clearly detected by our naked eyes. When pH is 5, the A_{525}/A_{700} ratio of the positive sample is slightly lower than the other positive samples. It is because low pH could reduce the surface charge density and promote the aggregation of AuNps. And when the pH is 9, the A_{525}/A_{700} ratio of the negative sample is slightly higher than the other negative samples, since the charge repulsion between AuNPs became stronger.[4] This indicates that our method works well when the pH value is ranging from 5 to 9, especially in the physical pH range of 6-8. The detection of PCR products using AuNPs may be influenced when pH value is lower than 5 or higher than 9.

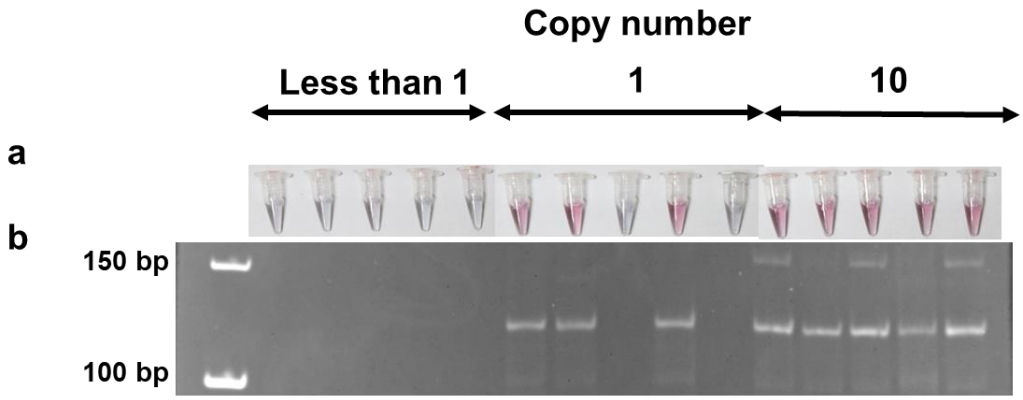

Fig. S8 Analysis of nested PCR products amplified from specimen contained low copies of the target gene. a), visual detection of the nest PCR products by this assay; b), gel analysis of the nest PCR products.

The specimen contained different copies (10 copies, 1 copy and less than 1 copy) was obtained by gradual dilution. The diluted specimen was amplified by nest PCR (45 cycles, combination of conventional PCR and indenting PCR). For each specimen, we conducted five individual replicates. The PCR products were analyzed by this assay and the gel electrophoresis, respectively.The detection results were all positive when the copy number was counted as 10. But there were only three positive results out of five samples when the copy number was counted as 1. This was because when the copies number was extremely low, it was difficult to guarantee that each specimen contained the same copy of the target gene just as counted. For the specimen supposed to contain 1 cpoy of target gene, there might be less than or more than 1 copy in the each specimen in reality. And when we continued to dilute the specimen, the counted copy number of the target gene was suppsed to be less than 1 copy, thus all the detection results were negative.

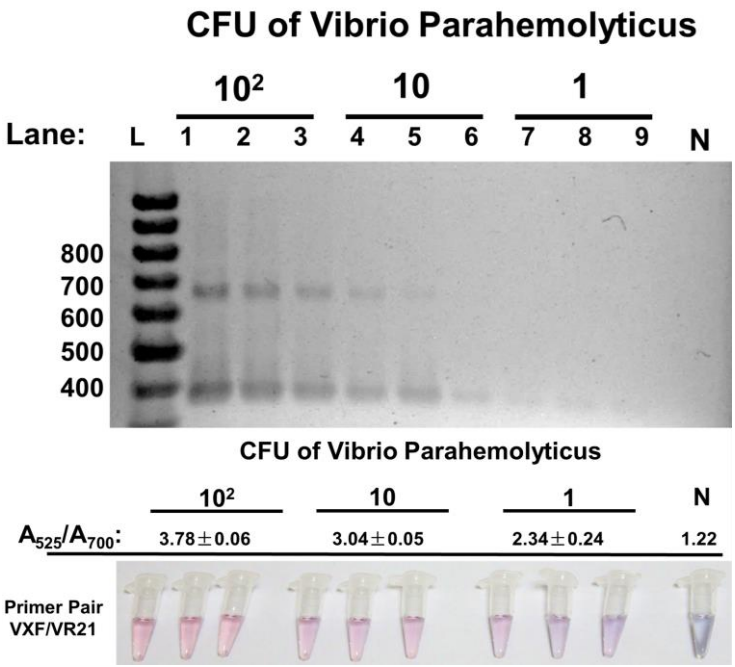

Fig. S9. Application of this assay in detection of Vibrio Parahemolyticus from polluted oysters. Estimated Colony-Forming Units (CFU) of Vibrio Parahemolyticus were added into oysters. The primer pair was targeted at the *tlh* gene (thermolabile hemolysin gene). The outer products are of 653 bp and the inner products are of 357 bp attached with 16 nt overhangs. Azobenzene modified primer VXF39 was used as indenting primer.

As shown in Fig. S9, gel analysis of PCR products demonstrates that there was no non-specific products, and all the samples containing Vibrio Parahemolyticus were detected as positive through naked eyes. The sensitivity was 1 CFU of Vibrio Parahemolyticus per 30 μg oysters.

Tab. S3 Oligonucleotides used for detection experiments in Fig. S9

ODNs	Sequence (5'→3')	Length/nt
VXF39	CCATGTCTAAAACCAA$_X$A$_X$GTTGATGACACTGCCAGATGC	39
VF21	GTTGATGACACTGCCAGATGC	21
VR21	TGAGTTGCTGTTGTTGGATGC	21
RT15	TGGTTTTAGACATGG-SH	15

References

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