Electronic Supplementary Material (ESI) for Analyst

Supporting Information

1. Sequences

2. Experimental Section

2.1 Isolation of HIV RNA and determination of the HIV RNA concentration

2.2 Colorimetric result of stand-displacement based signal amplification with different concentrations of segment a

2.3 Optimization of the concentration of Mg2+ of G-quadruplex stand-displacement based signal amplification

2.4 Optimization of reaction time of the stand-displacement based signal amplification

2.5 Colorimetric detection of HIV RNA

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1. Sequences

Identity	Sequences
HIV target sequence	5'- TTT GGA AAG GAC CAG CAA AGC TCC TCT GGA AAG GTG AAG GGG CAG TAG TAA TAC AAG ATA ATA GTG ACA TAA AAG TAG TGC CAA GAA GAA AAG CAA AGA TCA TTA GGG ATT ATG GAA AAC AGA TGG CAG GTG AT
F-primer	5'- TTT GGA AAG GAC CAG CAA A
R-primer	5'- ATC ACC TGC CAT CTG TTT TCC
Probe-1	5'- CTA CTT ATC TGA TGA ATG CTT TTC TTC TTG GCA CTA CTT TTA TGT CA AAC AGT AAC
Probe-2	5'- TTT CCC AAC CCG CCC TAC CCA C CTC AGC AGCATT CAT CAG ATA AGT AG
Segment a	5'- CTACTTATCTGATGAATG
CatG4	5'- TGA GGT GGG TAG GGC GGG TTG GGA AA

Table S1. Template, primers and probe sequences used in this study.

2. Experimental Section

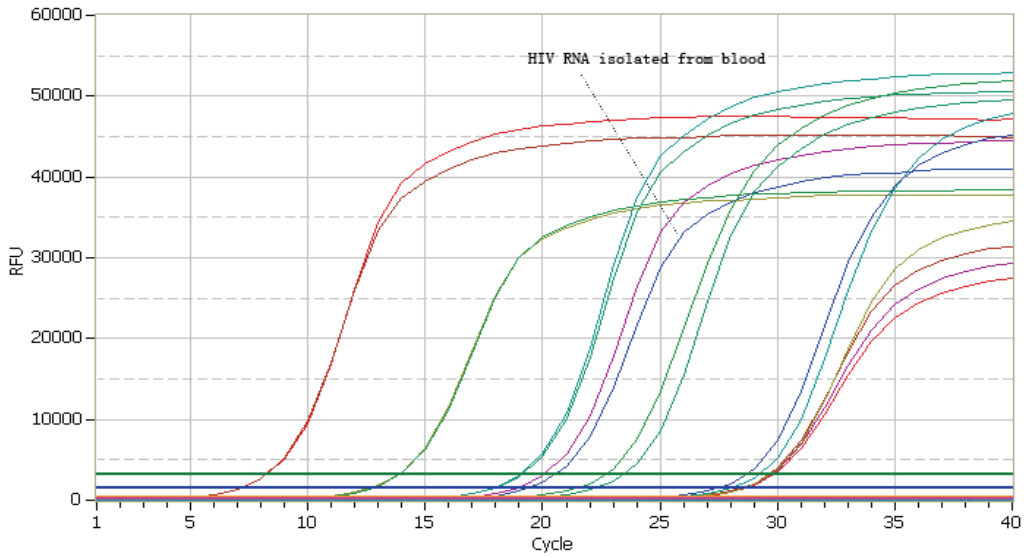
2.1 Isolation of HIV RNA and determination of the HIV RNA concentration

The TRIzol extraction method (Invitrogen, Carlsbad, CA, USA) was used here for the isolation of HIV-1 RNA from blood sample. Then the RT-PCR was performed in the presence of HIV RNA, 0.5μM forward primer, 0.5μM reverse primer, 200 μM dNTP, 5 units of TaqM1 polymerase, 1×Taq Buffer (20mM Tris-HCl (pH 8.4), 20mM KCl, 10mM $(NH_4)_2SO_4$, 2mM $MgSO_4$), DEPC water was added to the 50μL final volume. PCR procedure was as follows: 65℃ for 15 min; 95℃ for 1 min; 95℃ 20s, 51℃40s, 68℃ 40s, 30 cycles. After ligation of PCR product with vector, transfomation to E.coli and clone, the HIV cDNA plasmids were extracted and determined the concentration by an UV spectrophotometer.

Because the RNA isolated from blood included both HIV RNA and blood cell genome RNA, UV can't assay the HIV RNA concentration correctly, and thus we used real time reverse transcription qPCR to quantify it. HIV cDNA plasmids were transcribed to RNA which was determined the concentration by UV and was using as the standard sample for qPCR.

Real time reverse transcription qPCR was performed 1μl HIV RNA, 1μl 10×sybr green I, 0.5μM forward primer, 0.5μM reverse primer, 200 μM dNTP, 2.5 units of TaqM1 polymerase, 2.5μl 1x Taq Buffer (20mM Tris-HCl (pH 8.4), 20mM KCl, 10mM $(NH_4)_2SO_4$, 2mM $MgSO_4$), DEPC water was added to the 25μL final volume. PCR procedure was as follows: 65℃ for 15 min; 95℃ for 1 min; 95℃ 20s, 51℃40s, 68℃ 40s, 30 cycles.

A



B

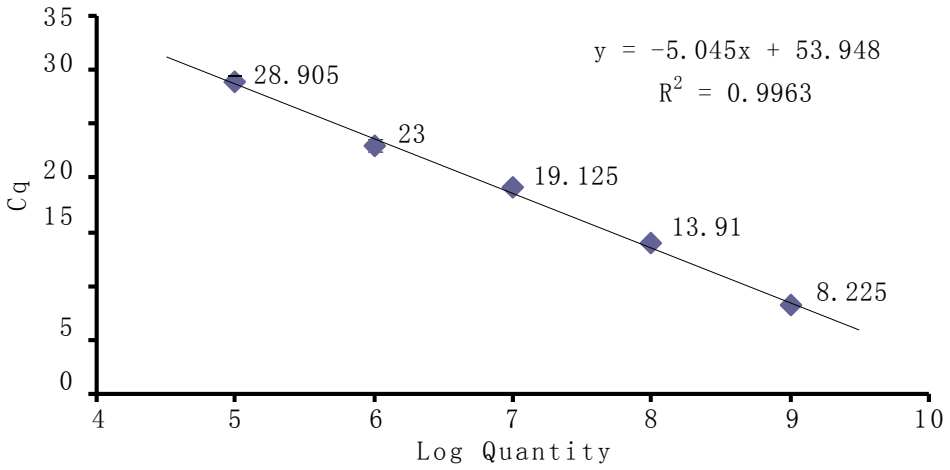


Figure S1. A is real time fluorescence curves of standard samples in five gradient concentrations (10^9, 10^8, 10^7, 10^6, 10^5 copies) of HIV RNA. From the standard curve (B), we can calculate the concentration of diluted HIV RNA samples isolated from blood is 8.6pM.

2.2 Colorimetric result of strand-displacement based signal amplification with different concentrations of DNA fragment T

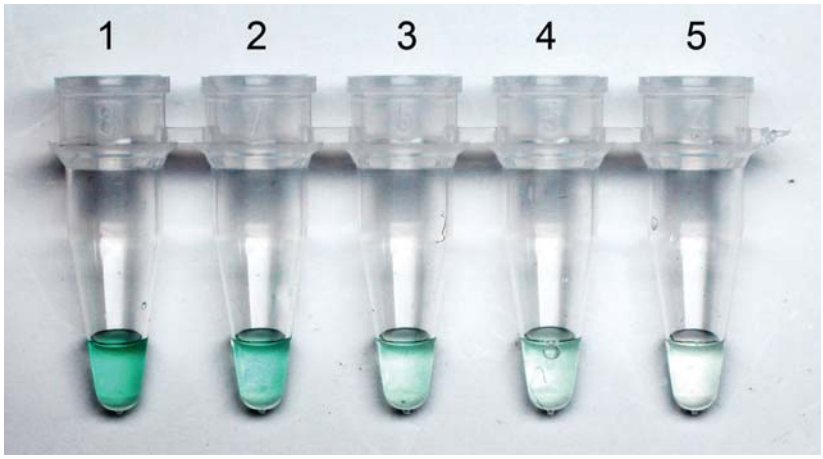


Figure S2. 0.3 μM Probe2 ,different concentrations of T (tube 1-5: containing 0.125uM, 0.05uM, 0.025uM, 0.0125uM, 0uM of DNA fragment **T**), 5 unit Bsm DNA Polymerase ,8 unit nicking enzyme were added into 1×Taq Buffer (20mM Tris-HCl (pH 8.4)，20mM KCl，10mM $(NH_4)_2SO_4$，2mM $MgSO_4$) at 37℃ for 2h. All samples were denatured at 95℃ for 1 min, and then hemin (1.2μM), $ABTS^{2-}$ (3.8mM), and H_2O_2 (1.5mM) were added.

2.3 Optimization of the concentration of Mg^{2+} of strand-displacement based signal amplification

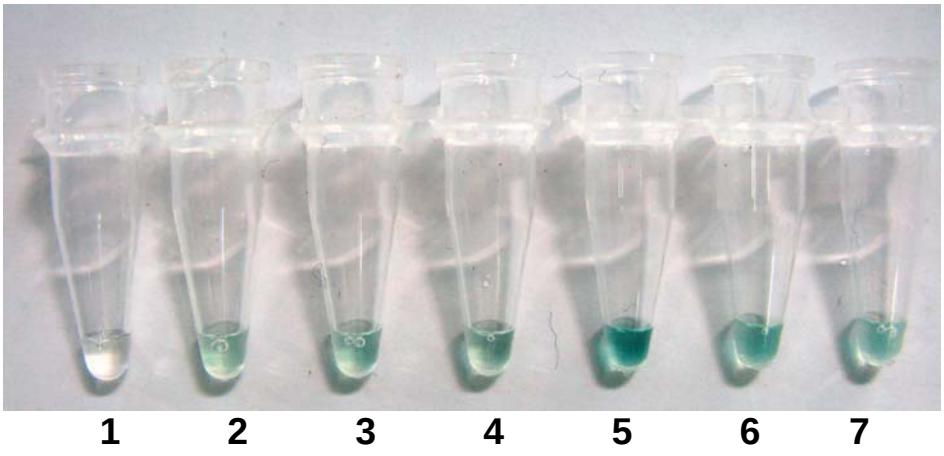


Figure S3. 0.3 μM Probe 2, 0.0125uM fragment **T**, 5 unit Bsm DNA Polymerase ,8 unit nicking enzyme and 1×Taq Buffer (20mM Tris-HCl (pH 8.4), 20mM KCl, 10mM $(NH_4)_2SO_4$, different concentrations of $MgSO_4$ (tube 2-7: containing 4mM, 6mM, 8mM, 10mM, 12mM, 14mM of $MgSO_4$) were added, 37℃ 2h. Samples were denatured at 95℃ for 1 mins and then hemin (1.2μM), $ABTS^{2-}$ (3.8mM), and H_2O_2 (1.5mM) were added.

2.4 Optimization of reaction time of the strand-displacement based signal amplification

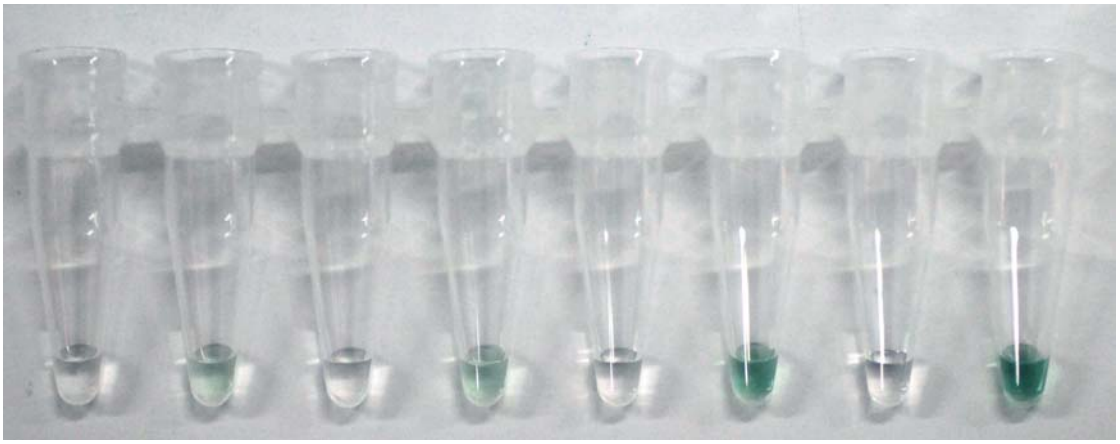


Figure S4. Dfferent reaction time(1 and 2: 0.5h, 3 and 4: 1h, 5 and 6: 1.5h, 7 and 8: 2h) of the G-quadruplex signal amplifications were performed on 37℃. .3 μM Probe2, 0.0125uM segment a, 5 unit Bsm DNA Polymerase ,8 unit nicking enzyme and 1×Taq Buffer (20mM Tris-HCl (pH 8.4), 20mM KCl, 10mM $(NH_4)_2SO_4$, 10mM $MgSO_4$) were added in 2,4,6,8 and 1, 3, 5, 7 is without segment a as negtive control. Samples were denatured at 95℃ for 1 mins and then hemin (1.2μM), $ABTS^{2-}$ (3.8mM), and H_2O_2 (1.5mM) were added. For followed reaction, We choosed 1.5h to be the suitable reaction time.

2.5 Colorimetric detection of HIV RNA

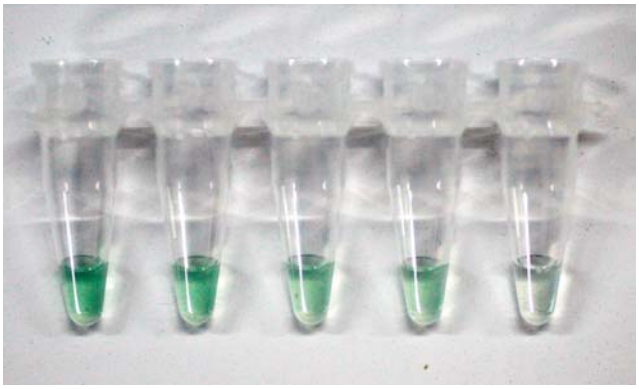


Fig.S5. Colorimetric detection of reaction product of different concentration of target HIV RNA after hemin, ABTS and H_2O_2 were added. From left to right: containing 600pM, 60pM, 6pM, 600fM, 0M of HIV RNA, respectively.