Supporting Information

for

A *One-Step* Label-Free Optical Genosensing System for Sequence-Specific DNA Related to the Human Immunodeficiency Virus Based on the Measurements of Light Scattering Signals of Gold Nanorods

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PREPARATION OF HIV-1 LTR REAL SAMPLES

1. HIV DNA isolation and construction of LTR sequence containing clones

The HIV-1 positive blood was obtained from a child in Huangpi district, Wuhan, Hubei (Corresponding HIV-1 B' isolates name and the full-length sequence accession in GenBank are 05CNHB_hp and DQ990880, respectively). The patient was infected with HIV-1 from his mother who was infected in turn with HIV-1 *via* a commercially paid blood donation. Template DNA for PCR was prepared from infected PBMCs using QIAamp® DNA Mini Kit (QIAGEN, GmbH, Germany). The 5' fragment (1.8 kb) extended from the 5' LTR to Gag region and was amplified using the primer pair LTRf (5'-TGGAAGGGCTAATTTAC-3') and LTR1.8k (5'-TCATCATTTCTTCT

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AGTGTAGC-3') hybridizing at position 1812-1833 of the isolated virus 05CNHB_hp. PCR was conducted using the LA Taq PCR system (Takara, Dalian, China). PCR products were gel purified with the TOPO® gel purification kit (Invitrogen, CA, USA) and cloned into a TA vector TOPO®-XL-Vector (Invitrogen, CA, USA), and then sequenced.

By this method, we obtained a HIV PCR product of HB-hp3-LTR1.8, which is consist of 1840 bp, and the base at the site from 615 to 635 is perfectly matched with the probe ssDNA. Next, employing restriction endonuclease reaction, we got 314 bp sequence fragment which contain the target ssDNA at the base site from the 615 to 635 and adjacent sequence. Then, these sequence fragments were denatured by boiling to obtain single-stranded molecule.

2. Design the restriction enzyme site in HIV-1 LTR sequence

The restriction enzyme site in HIV PCR products HB-hp3-LTR1.8 (1840 bp) was designed by the software Victor NTI, including three HindIII sites (the 530-535 bp, the1081-1086 bp and 1710-1715 bp) and one EcoRI site (the 838-842 bp). If digested by HindIII and EcoRI enzyme, the long sequence of HB-hp3-LTR1.8 should produce 5 fragments of 535 bp, 314 bp, 249 bp, 636 bp and 120 bp and among them the 314bp fragment contains the target sequence (the 615-631 bp).

3. Restriction endonuclease reaction

The restriction endonuclease system and corresponding control experiment of enzymes was incubated at 37 ℃ for 4 h as Table S1 shows. The 25 μL restriction digestion system consisted of 1 μL PCR products (207.5 μg/mL), 2.5 μL 10×Buffer, 1 μL HindIII enzyme (15 U/μL) and 1 μL EcoRI (15 U/μL) enzyme and 19.5 μL ddH_2O.The 50 μL restriction digestion system consisted of 2 μL PCR products, 5 μL 10×Buffer, 2 μL HindIII enzyme and 2 μL EcoRI enzyme and 39 μL ddH_2O. The corresponding control group of enzymes doesn't contain DNA.

Table S1 the enzymatic system

	Total Volume (μL)	ddH_2O (μL)	Buffer (μL)	DNA (μL)	HindIII (μL)	EcoRI (μL)
system 1	25	19.5	2.5	1	1	1
system 2	50	39	5	2	2	2
control group	50	41	5	0	2	2

Conditions: DNA, 207.5 μg/mL; Hind□, 15 U/μL; EcoRI, 15 U/μL; restriction time, 4h; temperature, 37□

4. Gel electrophoresis analysis

The two restriction digestion systems were analyzed by agarose gel electrophoresis. As Fig.S1 shows, two weak straps nearby 300bp represent fragments of 314 bp and 249 bp respectively, and two straps in the middle represent fragments of 535 bp and 636 bp, moreover, the suprema strap represents whole DNA sequence that wasn't digested by enzymes. The strap of 120 bp fragments theoretically was not observed perhaps because of the low content. In conclusion, the presence of strap of 314 bp indicates that we have successfully obtained the short fragment which contains the target sequence.

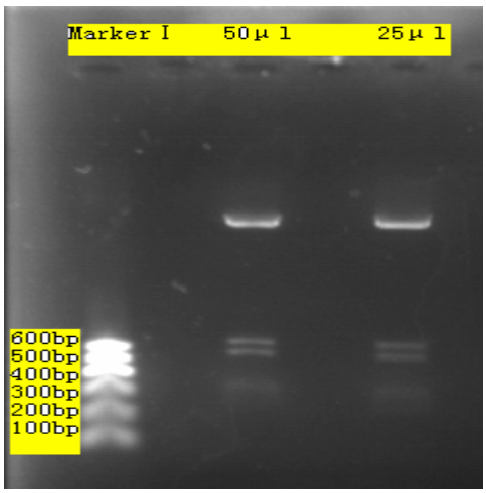

Fig. S1. Gel electrophoresis (1% agarose gel at 100 V, 45mA, 25 min) was used to confirm short DNA sequence fragment from HIV PCR products HB-hp3-LTR1.8 after restriction endonuclease reaction.