## Supporting Information

# DNAzyme-Triggered Equilibrium Transfer with Self-Activated CRISPR Cas12a Biosensor Enables One-Pot Diagnosis of Nucleic Acids

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**Materials and reagents**

Oligonucleotides used in this article (Table S1), RNase inhibitor, RNase-free $H_2O$, T4 polynucleotide kinase, DNase Ⅰ, and Exonuclease Ⅰ were bought from Sangon Biotech Co., Ltd. (Shanghai, China). HIV-1 and HIV-2 pseudoviruses were purchased from Tsingke Biotechnology Co., Ltd. (Hangzhou, China). Recombinant LbCas12a (Cpf1) and CRISPR reporting test strips were obtained from FasTech Biotechnology Co., Ltd. (Hangzhou, China). T4 DNA ligase and T4 RNA ligase 2 were purchased from New England Biolabs Co., Ltd. (Ipswich, USA). Other chemical reagents used in this article were of analytical grade and bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). OI pathogens, including invasive fungi (*Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*) and bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) were obtained from the School of Medicine of Zhejiang University.

***In-vitro* preparation of nucleic acids**

The double-stranded DNAs (dsDNAs) mimicking the target fragments were prepared through annealing of two synthesized single-stranded DNA (ssDNA). Briefly, 10 μL 10 mM of each ssDNA was thoroughly mixed and incubated at 95 ℃ for 10 min, followed by cooling at a rate of 0.1 ℃/s until 4 ℃. The obtained dsDNA was diluted to each desired concentration and stored at -80 ℃ for further use. What's more, the hybridization of DNAzyme and different blockers was also operated through the same annealing program.

**Sample pretreatment and extraction**

Simulated samples were prepared by spiking various concentrations of HIV-1 pseudovirus into bovine plasma, which were extracted using BeaverBeads Viral DNA/RNA kit purchased from Beaver Co., Ltd. (Suzhou, China). Following the kit manual, each 200 μL sample was mixed with 40 μL proteinase K, 400 μL lysis buffer, 1 μL of carrier RNA, 200 μL of isopropanol, and 20 μL of magnetic beads in order. The mixture was thoroughly mixed and then incubated at 55°C for 15 min. Subsequently, the mixture was placed on a magnetic separator for 1 min to remove the supernatant. After two-step purification with washing buffer, the beads were air-dried at room temperature. Finally, 20 μL RNase-free $H_2O$ containing 20 U RNase inhibitor was thoroughly mixed with the beads and incubated at 55°C for 10 min. The mixture was then placed on a magnetic separator for 1 minute to collect the supernatant. The extracted samples were analyzed directly or stored at -80 ℃ for further

use.

**Conventional RPA and RT-RPA assay in two-step detection**

Following the manual of (RT)-RPA kit obtained from FasTech Biotechnology Co., Ltd. (Hangzhou, China), enzyme mixtures were dissolved by 25 μL reaction buffer, and then mixed with 2 μL 10 μM forward primer, 2 μL 10 μM reverse primer, 10 μL sample, and 8.5 μL RNase-free $H_2O$. Subsequently, 2.5 μl activation buffer was added to induce the reaction at 37 °C for 30 min. The amplification products were used for a two-step CRISPR assay directly or purified by the phenol-chloroform method before being analyzed by 3% agarose gel electrophoresis.

**Conventional fluorescence assay based on Cas12a in two-step detection**

A 20 μL optimized fluorescence assay based on Cas12a in two-step detection was composed of 50 nM LbCas12a, 150 nM crRNA, 20 U RNase inhibitor, 2 μL mimic target dsDNA or (RT-)RPA products, 750 nM fluorescence reporter as *trans*-cleavage substrate of Cas12a, 1× Cas12a reaction buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/ml recombinant albumin, pH 7.9), and RNase-free $H_2O$ added to make up the remaining volume. The mixture was thoroughly mixed and transferred to 384-well plates for incubation at 37 °C. The fluorescence signal of each test was measured by Varioskan Flash Multimode Plate Reader from Thermo Fisher Scientific Co., Ltd. (Waltham, USA) every 3 min ($\lambda_{Ex}$: 535 nm, $\lambda_{Em}$: 556 nm).

**Preparation of L-crRNA and C-crRNA**

The conventional linear crRNA (L-crRNA) of Cas12a was synthesized using an *in-vitro* transcription kit purchased from Thermo Fisher Scientific Co., Ltd. (Waltham, USA). Following the kit manual, each 20 μL transcription system consisted of 4 μL transcription buffer, 2 μL enzyme mixture, 8 μL 10 μM dNTPs, and 6 μL 5 μM dsDNA template with a T7 promoter. After reaction at 37 ℃ for 8 h, the transcription products were treated by DNase Ⅰ and further purified by RNA Clean & Concentrator kit purchased from Zymo Research Co., Ltd. (Orange County, USA). After quantification, the well-prepared L-crRNA was stored at -80 ℃ for further use.

To prepare circular-crRNA (C-crRNA), L-crRNA and substrate strand (substrate for ligation in Table S1) were phosphorylated at 5' end, respectively. Each 50 μL phosphorylation system consisted of 1× T4 polynucleotide kinase (PNK) buffer, 20 U T4 PNK, 40 U RNase inhibitor, 30 μL 10 μM L-crRNA or substrate strand, and RNase-free $H_2O$ added to make up the remaining volume. After the

incubation at 37 °C for 30 min, the mixtures were immediately heat-inactivated at 85 °C for 20 min. Subsequently, the one-step ligation system consisted of 1× ligation buffer, 40 μL phosphorylated C-crRNA, 40 μL phosphorylated substrate strand, 4 μL 100 μM DNA ligation template (Ligation template 1 in Table S1), 4 μL 100 μM RNA ligation template (Ligation template 2 in Table S1), 30 μL 50% PEG8000, 10 μL 100 mM $MgCl_2$, 5 μL 10 mM ATP, 60 U RNase inhibitor, and RNase-free $H_2O$ to make up the volume to 148 μL. After incubation at 65 ℃ for 10 min, the mixture was gradually cooled at a rate of 0.1 ℃/s until room temperature. Then, 100 U T4 DNA ligase, 100 U T4 RNA ligase 2, and RNase-free $H_2O$ were added to make up the final volume to 160 μL. After incubation at 25 °C for 4 h, the mixture was further treated by 320 U Exonuclease Ⅰ at 37 °C for 1 h, followed by heat-inactivation at 85 °C for 5 min. Then, the obtained C-crRNA was further purified by 15% denaturing urea PAGE and a ZR small-RNA PAGE Recovery kit purchased from Zymo Research Co., Ltd. (Orange County, USA). After quantification, the well-prepared C-crRNA was stored at -80 ℃ for further use.

**Detection procedure of qRT-PCR for HIV-1 diagnosis**

The spiked samples were extracted according to the same procedure as DESCRIBER assays, and then analyzed by a qRT-PCR kit (HiScript Ⅲ $U^+$ One Step) from Vazyme Co., Ltd. (Nanjing, China). Following the kit manual, each 20 μL reaction system consisted of 1× Reaction Mix, 1× Rox Reference Dye, 0.2 μM primer mix (qRT-PCR-FP and qRT-PCR-RP in Table S1), 0.1 μM Taqman probe (qRT-PCR-Probe in Table S1), 10 μL extracted sample, and RNase-free $H_2O$ added to make up the remaining volume. The corresponding qRT-PCR program was operated in StepOnePlus Real-Time PCR Systems from Thermo Fisher Scientific Co., Ltd. (Waltham, USA), which included (1) reverse transcription at 55 °C for 10 min, (2) pre-denaturation at 95 °C for 30 s, (3) 45 cycles of denaturation at 95 °C for 10 s and extension at 60 °C for 30 s. The fluorescence intensity was measured after extension in each cycle, and the thresholds were set at ten times the standard deviation automatically recorded in cycles 3~15.

**Table S1.** Important abbreviations used in the paper

| Abbreviations | Full names or meanings |
|---|---|
| DESCRIBER | DNAzyme-triggered equilibrium transfer with self-activated CRISPR Cas12a biosensor |
| POCT | Point-of-care testing |
| HIV-1 | Human immunodeficiency virus - 1 |
| CRF | Circulating recombinant forms |
| cPPT/CTS | Central polypurine tract/central termination sequence |
| CRISPR | Clustered regularly interspaced short palindromic repeats |
| Cas | CRISPR-associated protein |
| PAM | Protospacer adjacent motif |
| crRNA | CRISPR RNA |
| L-crRNA | Linear crRNA |
| C-crRNA | Circular-crRNA |
| Reborn L-crRNA | C-crRNA treated by DNAzyme |
| LT | Ligation template |
| 2AP | 2-aminopurine-modified sites |
| Kd | Dissociation constant |
| RPA | Recombinase-polymerase amplification |
| RT-RPA | Reverse transcription-RPA |
| ssDNA | Single-stranded DNA |
| dsDNA | Double-stranded DNA |
| cDNA | Complementary DNA |
| FP | Forward primer |
| RP | Reverse primer |
| Key-shaped RP | A specific RP created by successively connecting the ends of the DNAzyme, the blocker 0831, and the normal RP sequences |

**Table S2.** Oligonucleotides used in this study

| Name | Sequences (5'-3') |
|---|---|
| His-dependent DNAzyme | TGAAACATCTCATAACGGGGCTGTGCGGCTAGGAAGTAGTGACCTCAGC |
| Substrate-FAM | FAM-<br>TTTCGTTCTGAGCCGCTGAGGTCACrAGGATGAGATGTTTCACGTTCCGAGTTT |
| Substrate reporter | TTTCGTTCTGAGCCGCTGAGGT(FAM)CACrAGGAT($BHQ_1$)GAGATGTTTCACGT<br>TCCGAGTTT |
| Blocker-0118 | CCGTTATGAGATGTTTCA |
| Blocker-0120 | CCCCGTTATGAGATGTTTCA |
| Blocker-0122 | AGCCCCGTTATGAGATGTTTCA |
| Blocker-0124 | ACAGCCCCGTTATGAGATGTTTCA |
| Blocker-0126 | GCACAGCCCCGTTATGAGATGTTTCA |
| Blocker-0128 | CCGCACAGCCCCGTTATGAGATGTTTCA |
| Blocker-0130 | AGCCGCACAGCCCCGTTATGAGATGTTTCA |
| Blocker-0132 | CTAGCCGCACAGCCCCGTTATGAGATGTTTCA |
| Blocker-0134 | TCCTAGCCGCACAGCCCCGTTATGAGATGTTTCA |
| Blocker-0136 | CTTCCTAGCCGCACAGCCCCGTTATGAGATGTTTCA |
| Blocker-0138 | TACTTCCTAGCCGCACAGCCCCGTTATGAGATGTTTCA |
| Blocker-1326 | GCACAGCCCCGTTA |
| Blocker-1227 | CGCACAGCCCCGTTAT |
| Blocker-1128 | CCGCACAGCCCCGTTATG |
| Blocker-1029 | GCCGCACAGCCCCGTTATGA |
| Blocker-0930 | AGCCGCACAGCCCCGTTATGAG |
| Blocker-0831 | TAGCCGCACAGCCCCGTTATGAGA |
| Blocker-0732 | CTAGCCGCACAGCCCCGTTATGAGAT |
| Blocker-0633 | CCTAGCCGCACAGCCCCGTTATGAGATG |
| Blocker-0534 | TCCTAGCCGCACAGCCCCGTTATGAGATGT |
| Blocker-0435 | TTCCTAGCCGCACAGCCCCGTTATGAGATGTT |
| Blocker-0336 | CTTCCTAGCCGCACAGCCCCGTTATGAGATGTTT |
| Blocker-0237 | ACTTCCTAGCCGCACAGCCCCGTTATGAGATGTTTC |
| RT-RPA primer $F_1$ | TACAGTGCAGGGGAAAGAATAGTAGACATA |
| RT-RPA primer $F_2$ | ATTGGGGGGTACAGTGCAGGGGAAAGAATA |
| RT-RPA primer $F_3$ | GTAGACATAATAGCAACAGACATACAAACT |
| RT-RPA primer $F_4$ | TCCACTTTGGAAAGGACCAGCAAAGCTCCT |
| RT-RPA primer $F_5$ | TTACAGGGACAGCAGAAATCCACTTTGGAA |
| RT-RPA primer $F_6$ | TTTTCGGGTTTATTACAGGGACAGCAGAAA |
| RT-RPA primer $R_1$ | TTTATGTCACTATTATCTTGTATTACTACT |
| RT-RPA primer $R_2$ | ATGTTCTAATCCTCATCCTGTTTTCTTGCC |
| Key-shaped $R_2$ | TGAAACATCTCATAACGGGGCTGTGCGGCTAGGAAGTAGTGACCTCAGCTAG<br>CCGCAC(Spacer)AGCCCCGTTATGAGATCTAATCCTCATCCTGTTTTCTTGCC |
| Key-shaped $R_2$ with 2AP-1 | TGAAACATCTCATAACGGGGC(2AP)GTGCGGCTAGGAAGTAGTGACCTCAGCT<br>AGCCGCAC(Spacer)AGCCCCGTTATGAGATCTAATCCTCATCCTGTTTTCTTGCC |

| | |
|---|---|
| Key-shaped $R_2$ with 2AP-2 | TGAAACATCTCATAACGGGGCTGTGCGGC(2AP)AGGAAGTAGTGACCTCAGCT AGCCGCAC(Spacer)AGCCCCGTTATGAGATCTAATCCTCATCCTGTTTTCTTGCC |
| Mimic target-F | ATTAGGGATTATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGAAAAC AG |
| Mimic target-R | CTGTTTTCTTGCCACACAATCATCACCTGCCATCTGTTTTCCATAATCCCTAAT |
| Trans-F | TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTAGATTTGCCACACAAT CATCACCT |
| Trans-R | AGGTGATGATTGTGTGGCAAATCTACACTTAGTAGAAATTACCCTATAGTGAG TCGTATTA |
| HIV-1 L-crRNA | UAAUUUCUACUAAGUGUAGAUUUGCCACACAAUCAUCACCU |
| Substrate for ligation | TTCTGAGCCGCTGAGGTCACrAGGATGAGATGTTTCACGTTCCGArG |
| Ligation template 1 | CGGCTCAGAAAGGTGATGAT |
| Ligation template 2 | GUAGAAAUUACUCGGAACGU |
| HIV-1 C-crRNA | UAAUUUCUACUAAGUGUAGAUUUGCCACACAAUCAUCACCUTTCTGAGCCG CTGAGGTCACrAGGATGAGATGTTTCACGTTCCGArG |
| Fluorescence reporter | HEX-NNNNNNNNNNNN-$BHQ_1$ |
| Test strip reporter | FAM-NNNNNNNNNNNN-Biotin |
| qRT-PCR-FP | ACAGCAGTACAAATGGC |
| qRT-PCR-RP | TCCCTGTAATAAACCCGAAAAT |
| qRT-PCR-Probe | FAM-ATTGGGGGGTACAGTGCAGGGGAA-MGB |

**NOTE:**

The ribonucleotide residues in substrate-FAM, substrate reporter, substrate for ligation, and HIV-1 C-crRNA were marked in red.

The spacer-modified sites in Key-shaped $R_2$, Key-shaped $R_2$ with 2AP-1, and Key-shaped $R_2$ with 2AP-2 were marked in purple.

The 2-aminopurine-modified sites in Key-shaped $R_2$ with 2AP-1, and Key-shaped $R_2$ with 2AP-2 were marked in yellow.

The crRNA segment and the substrate segment in HIV-1 C-crRNA were marked in blue and green, respectively.

To ensure the His-dependent DNAzyme was compatible with the reaction temperature of RPA and Cas12a (37°C), the lengths of the $C_1$ and $C_2$ regions in the original sequence were adjusted, and the corresponding DNAzyme activity was characterized by native PAGE analysis. As shown in Figure S1, in the presence of His, DNAzyme specifically cleaved the ribonucleotide residue in the substrate and performed well at 37°C. The cleavage activity of DNAzyme was progressively enhanced with the increased concentration of His, and 80 mM His could effectively activate DNAzyme under working conditions. Subsequently, different concentrations of His were added to the conventional RPA system and Cas12a fluorescence assay, respectively. As depicted in Figure S2, His with concentrations below 80 mM didn't significantly interfere with the activities of RPA and Cas12a, whereas 100 mM His seemed to slightly reduce RPA efficiency. Therefore, both His and the His-dependent DNAzyme were compatible with the RPA and Cas12a systems, and suitable for establishing "one-pot" detection systems.

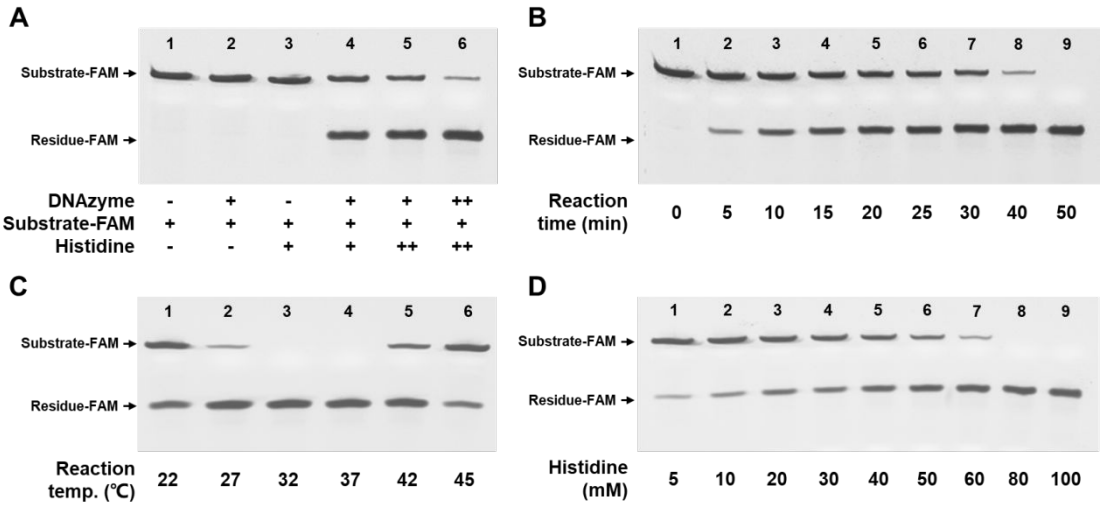



**Figure S1.** Characterization of the cleavage activity of histidine-dependent DNAzyme. (A) Characterization of the feasibility of histidine-dependent DNAzyme to cleave its substrate. (DNAzyme (-: 0, +: 100, ++: 200 nM), substrate-FAM (2 μM), histidine (-: 0, +:40 mM, ++: 80 mM), 37 ℃, 60 min.) (B) Characterization of reaction time on the performance of DNAzyme activity. (DNAzyme (500 nM), substrate-FAM (2 μM), histidine (80 mM), 37 ℃.). (C) Characterization of reaction temperature on the performance of DNAzyme activity. (DNAzyme (500 nM), substrate-FAM (2 μM), histidine (80 mM), 60 min.). (D) Characterization of histidine concentration on the performance of DNAzyme activity. (DNAzyme (500 nM), substrate-FAM (2 μM), 37 ℃, 60 min.).

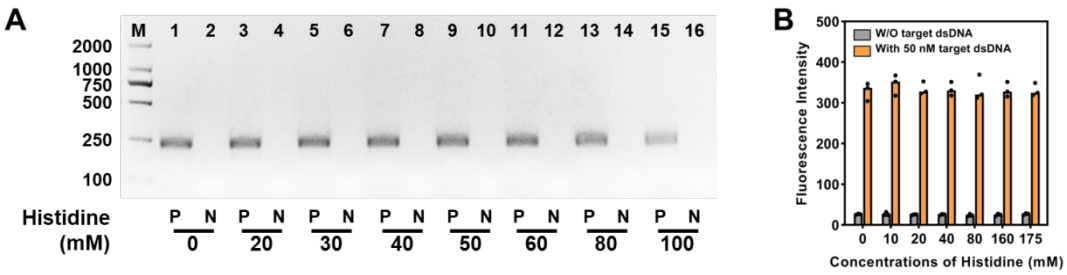



**Figure S2.** Characterization of histidine on the performance of RPA efficiency and Cas12a activity. (A) Characterization of RPA efficiency with different concentrations of histidine using agarose gel electrophoresis analysis. (P: positive tests in the presence of template; N: negative tests in the absence of template.). (B) Characterization of Cas12a activity using conventional fluorescence assay with different concentrations of histidine. The fluorescence intensity of each test was measured after 30 min reaction. (The points in histograms represented individual measurements, and the error bars indicate the mean values ± S.D.).

**A**

| (5'-3') | $C_1$ | Loop | $C_2$ |
|---|---|---|---|
| DNAzyme | TGAAACATCTCATAACGGGGCTGTGCGGCTAGGAAGTAGTGACCTCAGC | | |
| | 1 10 20 30 40 | | |
| Blocker-0118 | ACTTTGTAGAGTATTGCC | | |
| Blocker-0120 | ACTTTGTAGAGTATTGCCCC | | |
| Blocker-0122 | ACTTTGTAGAGTATTGCCCCGA | | |
| Blocker-0124 | ACTTTGTAGAGTATTGCCCCGACA | | |
| Blocker-0126 | ACTTTGTAGAGTATTGCCCCGACACG | | |
| Blocker-0128 | ACTTTGTAGAGTATTGCCCCGACACGCC | | |
| Blocker-0130 | ACTTTGTAGAGTATTGCCCCGACACGCCGA | | |
| Blocker-0132 | ACTTTGTAGAGTATTGCCCCGACACGCCGATC | | |
| Blocker-0134 | ACTTTGTAGAGTATTGCCCCGACACGCCGATCCT | | |
| Blocker-0136 | ACTTTGTAGAGTATTGCCCCGACACGCCGATCCTTC | | |
| Blocker-0138 (3'-5') | ACTTTGTAGAGTATTGCCCCGACACGCCGATCCTTCAT | | |

**B**

| (5'-3') | $C_1$ | Loop | $C_2$ |
|---|---|---|---|
| DNAzyme | TGAAACATCTCATAACGGGGCTGTGCGGCTAGGAAGTAGTGACCTCAGC | | |
| | 1 10 20 30 40 | | |
| Blocker-1326 | ATTGCCCCGACACG | | |
| Blocker-1227 | TATTGCCCCGACACGC | | |
| Blocker-1128 | GTATTGCCCCGACACGCC | | |
| Blocker-1029 | AGTATTGCCCCGACACGCCG | | |
| Blocker-0930 | GAGTATTGCCCCGACACGCCGA | | |
| Blocker-0831 | AGAGTATTGCCCCGACACGCCGAT | | |
| Blocker-0732 | TAGAGTATTGCCCCGACACGCCGATC | | |
| Blocker-0633 | GTAGAGTATTGCCCCGACACGCCGATCC | | |
| Blocker-0534 | TGTAGAGTATTGCCCCGACACGCCGATCCT | | |
| Blocker-0435 | TTGTAGAGTATTGCCCCGACACGCCGATCCTT | | |
| Blocker-0336 | TTTGTAGAGTATTGCCCCGACACGCCGATCCTTC | | |
| Blocker-0237 | CTTTGTAGAGTATTGCCCCGACACGCCGATCCTTCA | | |
| Blocker-0138 (3'-5') | ACTTTGTAGAGTATTGCCCCGACACGCCGATCCTTCAT | | |

**Figure S3.** Design of blockers to silence the activity of DNAzyme. (A) Blocker 0138~0118 were designed to progressively shorten from the 5' end to reduce complementarity with the loop region of DNAzyme. (B) Blocker 0138~1326 were designed to progressively shorten from both the 3' and 5' ends to reduce complementarity with the $C_1$ and loop regions of DNAzyme.

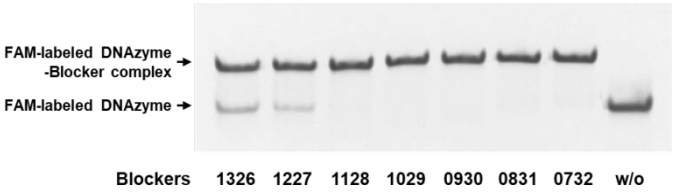



**Figure S4.** Characterization of molecular binding interactions between DNAzyme and blockers (1326~0732).

C-crRNA was prepared using a one-step reaction based on dual padlock probes (DNA ligation template (LT1) and RNA ligation template (LT2)), where 5'-phosphorylated L-crRNA and the substrate of the His-dependent DNAzyme were ligated by T4 DNA ligase and T4 RNA ligase 2. The reaction products were analyzed using denaturing urea PAGE electrophoresis, as depicted in Figure S5. Both the single- and double-ligase-mediated conjugates showed larger molecular weights compared to L-crRNA and the substrate. Following digestion with Exonuclease Ⅰ (EXO Ⅰ), the double-ligase-mediated conjugates retained distinct electrophoretic bands, indicating successful crRNA circularization through this method. After purification by PAGE, the C-crRNA was utilized for subsequent characterization of its interactions with Cas12a.

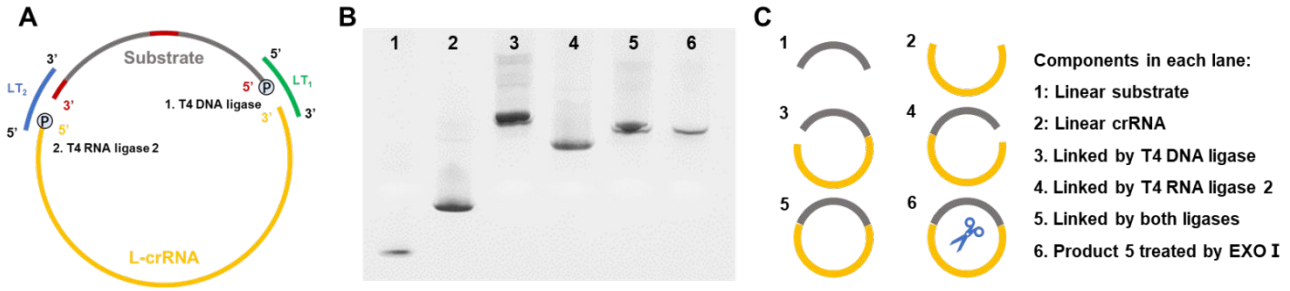



**Figure S5.** Characterization of C-crRNA prepared by one-step ligation. (A) Schematic of the C-crRNA preparation method. L-crRNA (the yellow strand) and substrate of DNAzyme (the gray strand with ribonucleotide residues marked in red) were ligated by T4 DNA ligase and T4 RNA ligase 2 with the assistance of DNA ligation template ($LT_1$, the green strand) and RNA ligation template ($LT_2$, the blue strand). (B), (C) Characterization of reaction products analyzed using denaturing urea PAGE electrophoresis. (line 1: Substrate of DNAzyme; line 2: L-crRNA; line 3: T4 DNA ligase-mediated conjugate; line 4: T4 RNA ligase 2-mediated conjugate; line 5: Double-ligase-mediated conjugate; line 6: Double-ligase-mediated conjugate treated by EXO Ⅰ.).

Six pairs of amplification primers designed from the region were screened (Figure S6A). Since the $F_6R_2$ primer pair showed the best efficiency and no significant nonspecific amplification, $R_2$ was selected to construct the corresponding self-blocking key-shaped primer for further validation. Templates with different concentrations of the HIV-1 genome were amplified by RT-RPA using the $F_6$ and Key-shaped $R_2$. As shown in Figure S6B, the amplification products were characterized by agarose gel electrophoresis and could identify the HIV-1 genome with concentrations as low as 500 CPs/mL.

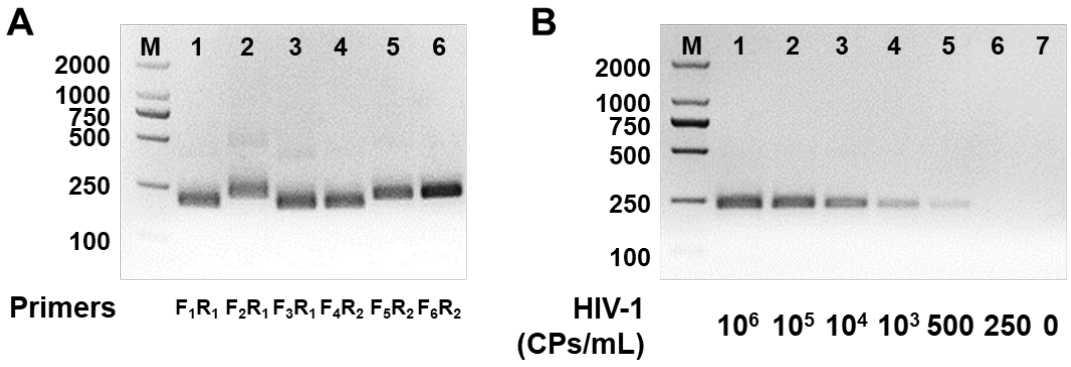



**Figure S6.** Screening of RT-RPA primers and characterization of amplification efficiency. (A) Six pairs of RT-RPA primers designed from the *pol* fragment containing the central polypurine tract/central termination sequence (cPPT/CTS) were screened by characterized by agarose gel electrophoresis. (B) Characterization of RT-RPA efficiency using the $F_6$ and Key-shaped $R_2$ in the presence of different concentrations of HIV-1 genome as amplification template.

Based on the sequence of the above amplification fragment, a mimic target dsDNA and its matching L-crRNA were prepared. The performance of this detection system was evaluated using a conventional Cas12a fluorescence assay. As shown in Figure S7A and S7B, the HIV-1 L-crRNA could activate the *trans*-cleavage activity of Cas12a in the presence of the mimic target, leading to the cleavage of the fluorescence reporter and a corresponding increase of fluorescence signal. Without nucleic acid amplification techniques, this Cas12a fluorescence assay system could detect mimic targets as low as 0.1 nM within 30 min. Moreover, the two-step RT-RPA-Cas12a system could achieve sensitive detection of 500 CPs/mL HIV-1 genome within 1 h (30 min for each step), demonstrating its potential for HIV-1 detection (Figure S7C and S7D).

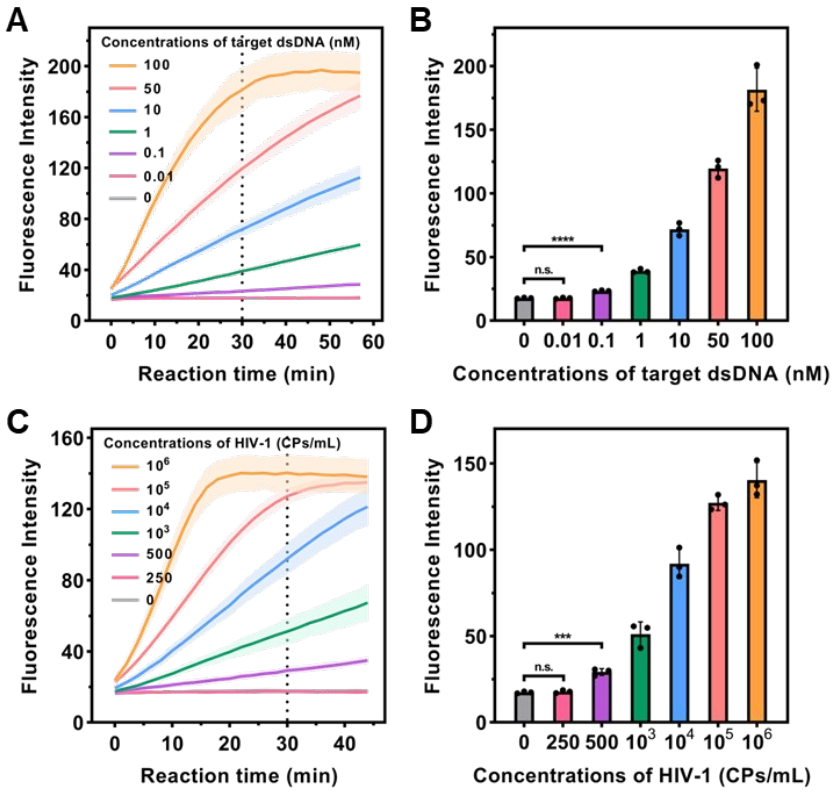



**Figure S7.** Sensitive of conventional fluorescence assay based on Cas12a. (A), (B) Reaction kinetics of detecting dsDNA mimic without nucleic acid amplification techniques and the fluorescence intensity measured at 30 min. (C), (D) Reaction kinetics of detecting HIV-1 genome with the help of RT-RPA and the fluorescence intensity measured at 30 min. (Experiments in each group were run in three technical replicates (n=3). Reaction kinetics curve represented means (bold line) ± S.D. (area fill). The points in histograms represented individual measurements, and the error bars indicate the mean values ± S.D. n.s.: no significance, ***: P<0.001, ****: P<0.0001 in Student's t test.).

Based on the kinetic curves shown in Fig. S8A and the linear relationship between fluorescence intensity changes and the amount of cleaved reporter (Fig. S8B), the rate of DESCRIBER signal output throughout the reaction was calculated. As shown in Fig. S8C, at high concentrations of mimic target dsDNA ($10^5$, $10^6$, $10^7$ aM), the reaction exhibited three distinct phases. The first phase was the lag phase, during which DESCRIBER primarily focuses on RPA to generate amplicons and L-crRNA, resulting in fewer activated Cas12a molecules and a slower signal output reaction rate. The more amplicons and L-crRNA were produced, the more Cas12a molecules were activated, leading to a gradual increase in the reaction rate. Since the final concentrations of Cas12a and the reporter in DESCRIBER were 200 nM and 1 μM, respectively, the reporter remained in excess even when Cas12a was fully activated. Once Cas12a became saturated, the reaction transitioned into a linear phase, where the reaction rate remained relatively constant for a period, thus tending towards a zero-order kinetic model. As the reporter was gradually consumed, it became the limiting factor, leading to the depletion phase, during which the reaction rate decreased and eventually approached zero. At lower concentrations of mimic target dsDNA ($10^2$ and $10^3$ aM), the reaction remained in the unsaturated Cas12a lag phase throughout the testing period.

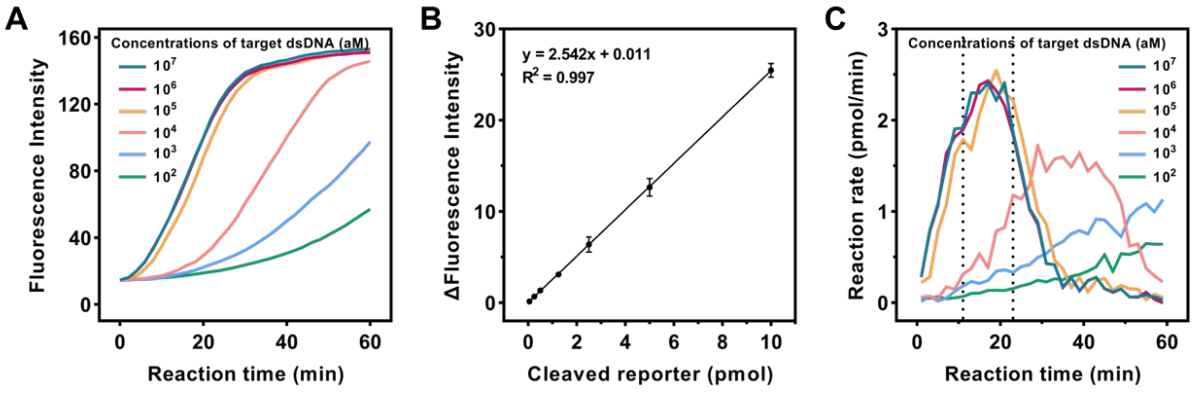



**Figure S8.** Kinetic analysis in the fluorescence assay based on DESCRIBER. (A) Reaction kinetics of detecting different concentrations of mimic target dsDNA using fluorescence assay based on DESCRIBER. (B) Linear relationship curve between fluorescence intensity changes and the amount of cleaved reporter. (C) Rate of the fluorescence assay based on DESCRIBER throughout the reaction. (Experiments in each group were run in three technical replicates (n=3). The reaction kinetics curve and reaction rate curve represented means. The error bars in the Linear relationship curve represented the mean values ± S.D.)

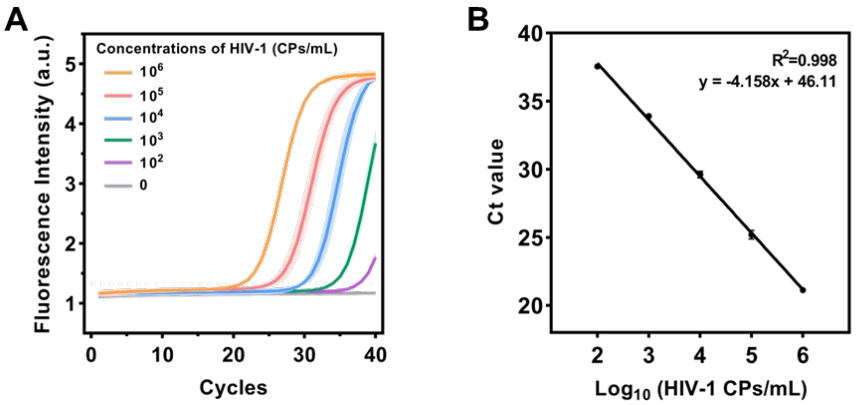



**Figure S9.** qRT-PCR assays for the comparison with DESCRIBER. A qRT-PCR system was established as the "Detection procedure of qRT-PCR for HIV-1 diagnosis" using primers and probe following the National Guideline for Detection of HIV/AIDS in China (2020). (A) Real-time fluorescence monitoring of HIV-1 with different concentrations ($10^6$, $10^5$, $10^4$, $10^3$, $10^2$, 0 CPs/mL) by qRT-PCR. (B) Standard working curve of qRT-PCR assay for HIV-1 diagnosis. ($Y = -4.158\times \log_{10}(X) + 46.11$, $R^2 = 0.998$, Y: the Ct values; X: the concentrations of HIV-1 (CPs/mL)). (Experiments in each group were run in three technical replicates (n=3). Real-time fluorescence monitoring curve represented means (bold line) ± S.D. (area fill). The error bars in the standard working curve represented the mean values ± S.D.)

**Table S3.** Detection results of spiked samples by DESCRIBER and qRT-PCR

| Sample information | | qRT-PCR | | | | | | | DESCRIBER | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Fluorescence assay | | | | | Test strip assay | | | | |
| Sample Num. | Spiked Conc. (CPs/mL) | $Ct_1$ | $Ct_2$ | $Ct_3$ | Ct Mean | Calculated Conc. (CPs/mL) | Recovery (%) | Judgement[a] | $FI_1$ | $FI_2$ | $FI_3$ | FI Mean | Judgement[b] | $TLI_1$ | $TLI_1$ | $TLI_1$ | TLI Mean | Judgement[c] |
| 1 | 100000 | 25.617 | 25.533 | 25.708 | 25.619 | 84724.740 | 84.72 | P | 141.635 | 144.107 | 140.815 | 142.186 | P | 6.114 | 6.327 | 5.720 | 6.054 | P |
| 2 | 700 | 34.572 | 34.710 | 34.663 | 34.648 | 570.865 | 81.55 | P | 32.450 | 31.581 | 34.795 | 32.942 | P | 0.950 | 1.211 | 1.241 | 1.134 | P |
| 3 | 0 | 41.174 | - | - | - | - | - | N | 15.588 | 15.633 | 15.140 | 15.454 | N | 0.487 | 0.438 | 0.477 | 0.467 | N |
| 4 | 5000 | 30.998 | 31.029 | 30.966 | 31.008 | 4286.618 | 85.73 | P | 73.565 | 76.141 | 75.691 | 75.132 | P | 3.340 | 3.310 | 3.207 | 3.286 | P |
| 5 | 0 | - | - | - | - | - | - | N | 15.485 | 15.279 | 15.112 | 15.292 | N | 0.497 | 0.476 | 0.478 | 0.484 | N |
| 6 | 3000 | 31.693 | 31.694 | 31.943 | 31.777 | 2800.078 | 93.34 | P | 64.382 | 69.224 | 63.984 | 65.863 | P | 2.396 | 2.151 | 2.408 | 2.318 | P |
| 7 | 0 | - | - | 41.024 | - | - | - | N | 15.541 | 15.342 | 15.427 | 15.437 | N | 0.407 | 0.509 | 0.412 | 0.443 | N |
| 8 | 9000 | 30.050 | 29.943 | 29.783 | 29.925 | 7805.779 | 86.73 | P | 107.558 | 105.759 | 102.605 | 105.307 | P | 3.668 | 4.026 | 3.899 | 3.864 | P |
| 9 | 0 | - | - | 41.251 | - | - | - | N | 15.255 | 15.610 | 15.209 | 15.358 | N | 0.488 | 0.428 | 0.430 | 0.449 | N |
| 10 | 50000 | 26.625 | 26.940 | 26.940 | 26.835 | 43215.76 | 86.43 | P | 127.218 | 131.43 | 135.325 | 131.324 | P | 4.805 | 5.029 | 4.451 | 4.762 | P |
| 11 | 7000 | 30.322 | 30.387 | 30.335 | 30.348 | 6176.818 | 88.24 | P | 90.419 | 95.617 | 92.867 | 92.968 | P | 3.027 | 3.659 | 3.180 | 3.289 | P |
| 12 | 0 | | - | 40.801 | - | - | - | N | 15.212 | 15.457 | 15.456 | 15.375 | N | 0.478 | 0.420 | 0.468 | 0.455 | N |
| 13 | 1000 | 33.971 | 34.057 | 33.862 | 33.963 | 834.2117 | 83.42 | P | 51.859 | 50.095 | 48.951 | 50.302 | P | 1.322 | 1.672 | 1.969 | 1.654 | P |
| 14 | 5000 | 31.09 | 30.935 | 30.998 | 30.998 | 4310.422 | 86.21 | P | 72.590 | 76.490 | 75.541 | 74.874 | P | 3.068 | 2.801 | 2.739 | 2.869 | P |
| 15 | 0 | - | - | 41.284 | - | - | - | N | 15.441 | 15.543 | 15.355 | 15.446 | N | 0.502 | 0.462 | 0.477 | 0.480 | N |
| 16[d] | 300 | 36.027 | 35.963 | 35.851 | 35.947 | 278.1019 | 92.7 | P | 15.331 | 15.484 | 15.416 | 15.410 | N | 0.485 | 0.418 | 0.489 | 0.464 | N |
| 17 | 500 | 35.219 | 35.023 | 34.985 | 35.076 | 450.5675 | 90.11 | P | 24.403 | 25.281 | 23.159 | 24.281 | P | 0.970 | 0.906 | 1.065 | 0.980 | P |
| 18 | 800 | 34.401 | 34.203 | 34.314 | 34.306 | 690.025 | 86.25 | P | 39.983 | 37.948 | 40.890 | 39.607 | P | 0.988 | 1.182 | 1.4811 | 1.217 | P |
| 19 | 900 | 34.105 | 33.964 | 34.257 | 34.109 | 769.7037 | 85.52 | P | 46.222 | 47.633 | 44.101 | 45.985 | P | 1.390 | 1.710 | 1.671 | 1.590 | P |
| 20 | 0 | - | 41.633 | - | - | - | - | N | 15.489 | 15.139 | 15.612 | 15.413 | N | 0.513 | 0.429 | 0.444 | 0.462 | N |
| 21 | 6000 | 30.502 | 30.583 | 30.670 | 30.585 | 5417.092 | 90.28 | P | 89.827 | 81.070 | 85.154 | 85.35 | P | 3.340 | 3.145 | 3.509 | 3.331 | P |
| 22 | 4000 | 31.294 | 31.339 | 31.236 | 31.290 | 3666.855 | 91.67 | P | 71.366 | 74.920 | 69.178 | 71.821 | P | 2.701 | 2.348 | 2.721 | 2.590 | P |
| 23 | 2000 | 32.478 | 32.613 | 32.622 | 32.571 | 1803.569 | 90.18 | P | 57.571 | 59.745 | 61.640 | 59.652 | P | 1.932 | 2.033 | 2.273 | 2.079 | P |
| 24 | 0 | - | - | - | - | - | - | N | 15.586 | 15.633 | 15.215 | 15.478 | N | 0.404 | 0.456 | 0.418 | 0.426 | N |
| 25 | 8000 | 30.033 | 30.105 | 30.096 | 30.078 | 7172.984 | 89.66 | P | 97.789 | 99.189 | 102.806 | 99.928 | P | 3.555 | 3.468 | 3.712 | 3.578 | P |
| 26[e] | 400 | 35.640 | 35.407 | 35.491 | 35.513 | 353.7213 | 88.43 | P | 16.418 | 17.048 | 17.4291 | 16.965 | P | 0.491 | 0.466 | 0.409 | 0.455 | N |
| 27 | 0 | - | - | - | - | - | - | N | 15.449 | 15.320 | 15.268 | 15.346 | N | 0.408 | 0.475 | 0.461 | 0.448 | N |
| 28 | 1000 | 33.814 | 33.692 | 33.759 | 33.755 | 936.2255 | 93.62 | P | 49.070 | 50.332 | 51.713 | 50.372 | P | 2.046 | 1.725 | 1.624 | 1.798 | P |
| 29 | 25000 | 27.959 | 28.118 | 27.885 | 27.987 | 22829.990 | 91.32 | P | 124.572 | 120.977 | 115.63 | 120.393 | P | 4.381 | 5.051 | 4.94 | 4.791 | P |
| 30 | 0 | - | - | | - | - | - | N | 15.375 | 15.279 | 15.353 | 15.336 | N | 0.454 | 0.468 | 0.46 | 0.461 | N |
| 31 | 600 | 34.784 | 34.561 | 34.704 | 34.683 | 560.011 | 93.34 | P | 27.564 | 28.395 | 29.157 | 28.372 | P | 0.836 | 1.174 | 0.959 | 0.990 | P |
| 32 | 500 | 35.150 | 35.276 | 35.093 | 35.173 | 426.925 | 85.38 | P | 23.877 | 23.335 | 24.870 | 24.027 | P | 0.693 | 0.567 | 0.862 | 0.707 | P |
| 33 | 10000 | 29.876 | 29.565 | 29.812 | 29.751 | 8596.931 | 85.97 | P | 105.971 | 109.515 | 102.871 | 106.119 | P | 4.041 | 4.257 | 4.533 | 4.277 | P |
| 34 | 0 | 41.779 | - | - | - | - | - | N | 15.589 | 15.434 | 15.144 | 15.389 | N | 0.464 | 0.417 | 0.474 | 0.452 | N |
| 35 | 75000 | 26.236 | 26.035 | 25.955 | 26.075 | 65817.620 | 87.76 | P | 135.128 | 140.016 | 141.205 | 138.783 | P | 5.644 | 5.107 | 4.736 | 5.162 | P |
| 36 | Blank | - | - | - | - | - | - | Blank | 15.154 | 15.460 | 15.343 | 15.319 | Blank | 0.459 | 0.510 | 0.478 | 0.482 | Blank |

**NOTE:**

[a] qRT-PCR categorized samples as "negative" if their Ct value exceeded 40 or was undetected; otherwise, they were deemed positive.

[b,c] The average value of the negative control (NTC) signal plus three times the standard deviation was used as the diagnostic cut-off line. Samples with signal values below the cut-off line were considered negative, while those above were deemed positive.

[d,e] For samples 16 and 26, the qPCR results differed from at least one DESCRIBER assay. This discrepancy was due to the concentrations of these two samples being 300 and 400 CPs/mL, which were close to the LOD of both DESCRIBER assays. However, since these concentrations were much lower than the threshold for preliminary diagnosis, the practical application value may not be affected.

**Table S4.** Cost analysis of DESCRIBER and qRT-PCR for HIV-1 diagnosis

| | Method | Manufacturer | Reagents cost ($/sample) | Concumables cost ($/sample) | Total cost without instruments ($/sample) | Instruments/ device cost ($) |
|---|---|---|---|---|---|---|
| 1 | qRT-PCR | Thermo Fisher/ TsingKe | Pretreatment kit (2.75) + qRT-PCR kit (5.50) + primers and probe (2.06) + others(0.27) = 10.58 | 1.11 | 11.69 | qRT-PCR thermocycler ( > 30000.00) |
| 2 | DESCRIBER | Our fluorescence assay | Pretreatment kit (2.75) + RT-RAA kit (2.75) + Cas12a/crRNA (1.10) + primers and reporter (2.48) + others(0.27) = 9.35 | 1.11 | 10.46 | Plate reader (2754.16) |
| 3 | | Our test strip assay | Pretreatment kit (2.75) + RT-RAA kit (2.75) + Cas12a/crRNA (1.10) + primers and reporter (2.48) + test strips (1.10) + others(0.27) = 10.45 | 1.11 | 11.56 | Thermocycler (27.54) |

**NOTE:**

This cost analysis of the fluorescence assay and the test strip assay based on DESCRIBER was calculated from the expenses at the research stage, according to our suppliers of reagents, consumables, and instruments.