Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Single-tube One-step Gel-based RT-RPA/PCR for Highly Sensitive Molecular Detection of HIV Virus

Naoki Uno[a], Ziyue Li[a,b], and Changchun Liu[a*]

[a] *Department of Biomedical Engineering, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030, United States*

[b] *Department of Biomedical Engineering, University of Connecticut, 260 Glenbrook Road, Storrs, CT 06029, United States*

[*] **Corresponding author**

Dr. Changchun Liu

Department of Biomedical Engineering

University of Connecticut Health Center

263 Farmington Avenue

Farmington, CT 06030

Phone: (860)-679-2565

E-mail: chaliu@uchc.edu

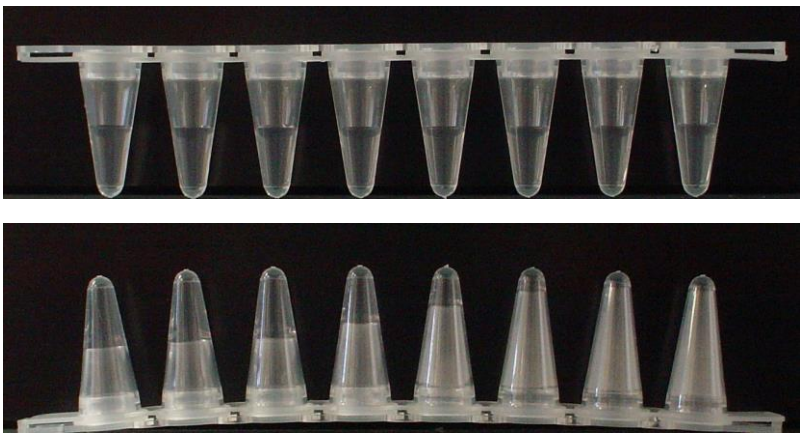

Figure S1. Melting temperature testing of 0.2% agarose gel. The 0.2% agarose gels were prepared in PCR tubes and incubated at the indicated temperature for 10 min. The PCR tubes were inverted after the incubation. Pictures before (top) and after (bottom) the inversion are shown.

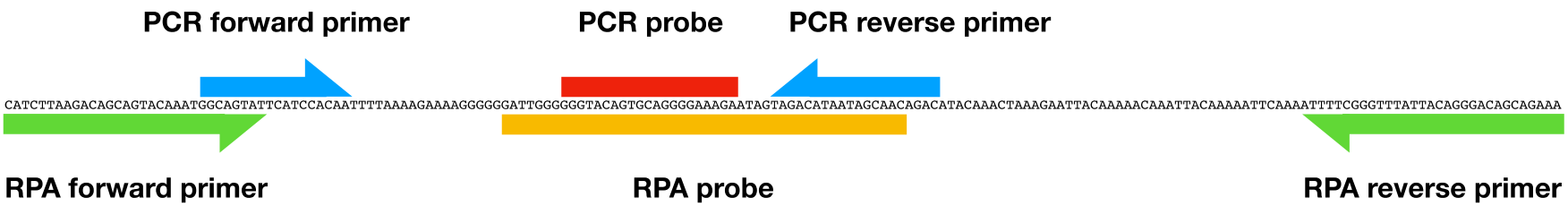

Figure S2. Locations of the primers and probes of the RPA and PCR reactions used in the study are shown on the HIV-1 reference genome.

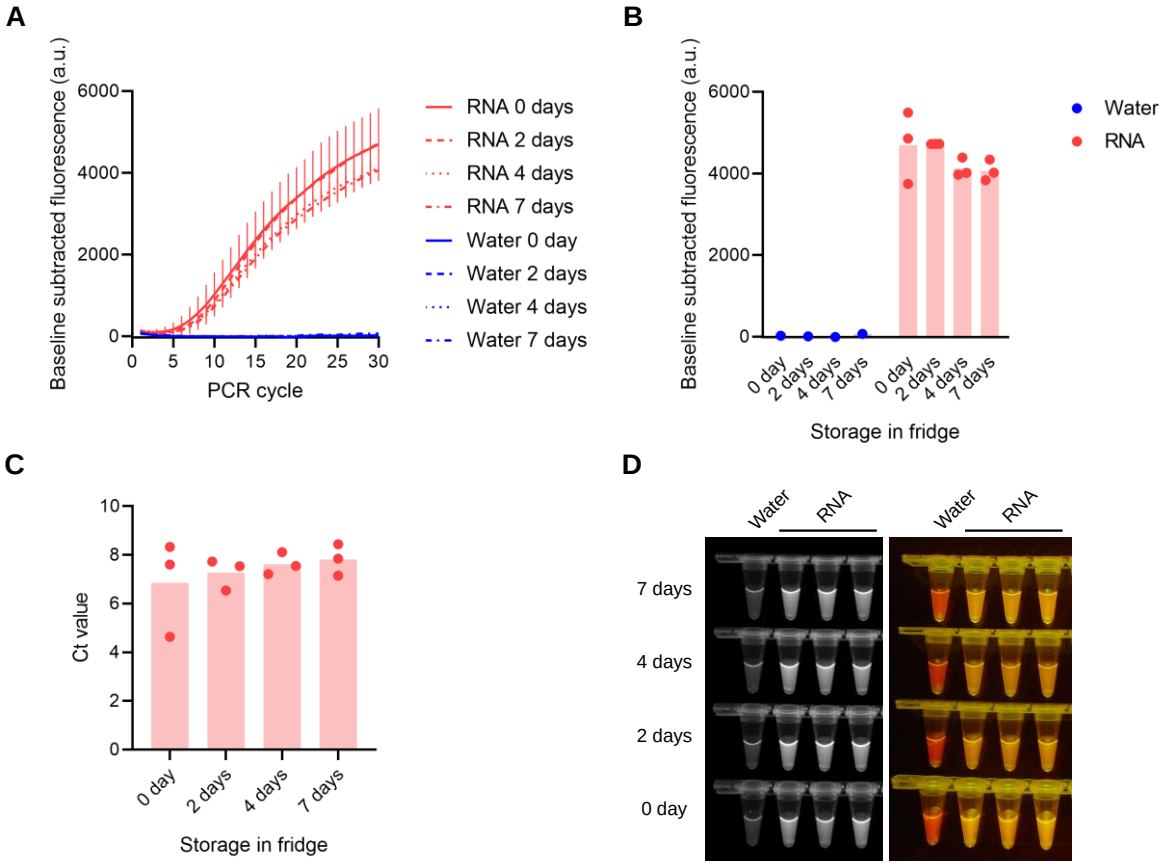

Figure S3. The PCR gel is stable at 4 ˚C for one week. SOG RT-RPA/PCR was carried out using PCR gels that were prepared in the same day or stored at 4 ˚C for 2 days, 4 days, or 7 days. (A) Realtime readouts. Error bars represent the mean ± standard deviation (n=3). (B) Fluorescence with mean at 30 cycles. (C) Cycle threshold (Ct) values with mean. (D) Endpoint fluorescence images taken by an imaging instrument (left) and LED transilluminator (right).

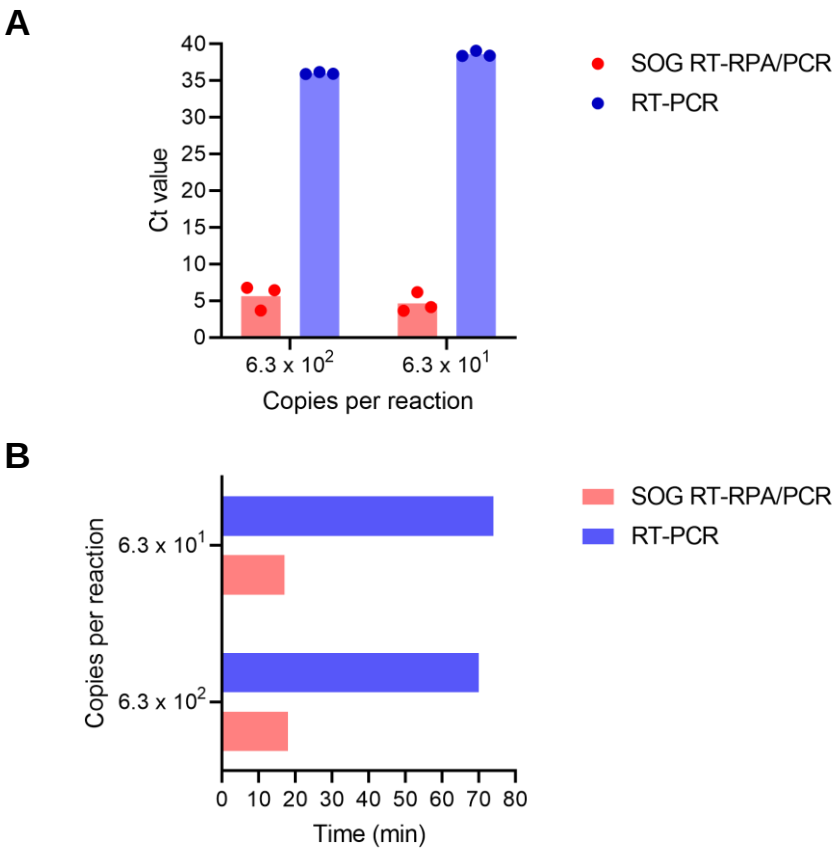

Figure S4. (A) Ct values of the real-time SOG RT-RPA/PCR and real-time RT-PCR in Figure 3A and D are shown with mean values (n=3). (B) The actual time required to reach to the mean Ct values for each method is shown.

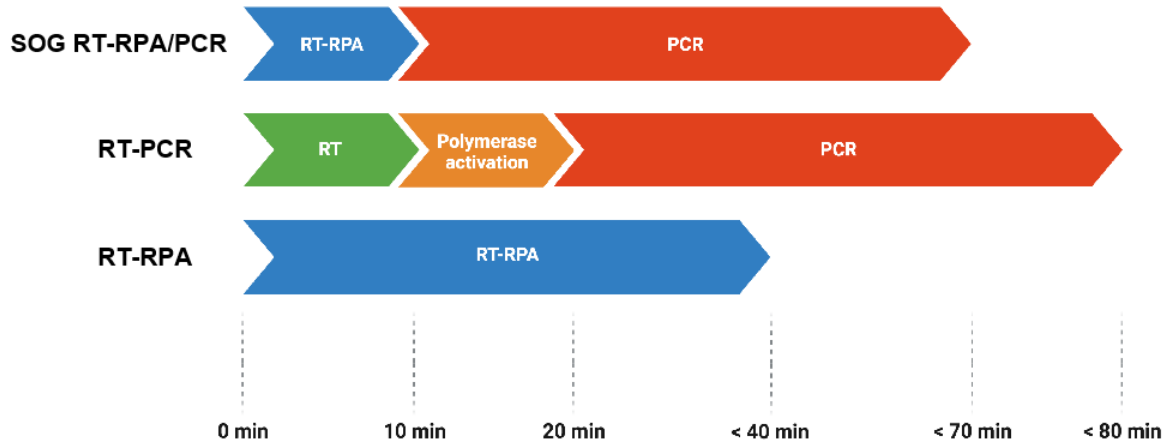

Figure S5. Schematic of the reaction time required for SOG RT-RPA/PCR, one-step real-time RT-PCR, and real-time RT-RPA. The reaction time required for one-step real-time RT-PCR is illustrated according to the manufacture's protocol. The PCR reaction time is shown based on a 40-cycle run.

Table S1. Oligonucleotide sequences used in the study

Oligonucleotide name	Sequence (5'-3')
RPA forward primer	CAYCTTAAGACAGCAGTACAAATGGCAGTAT
RPA reverse primer	TCTCTGCTGTCYCTGTAATAAACCCGRAAAT
PCR forward primer	GGCAGTATTCATTCACAA
PCR reverse primer	GTCTGTTGCTATTATGTCTA
PCR probe	FAM/TCTTTCCCC/ZEN/TGCACTGTACCC/IABkFQ
RPA probe	GATTGGGGGGTACAGTGCAGGGGAAAGAA/iFluorT//idSp/R/iBHQ-1dT/AGAYATAATAGCAAC/3SpC3