Supplementary Materials

for

# Quantitative evaluation of very low levels of HIV-1 reverse transcriptase by a highly sensitive RT-qPCR assay

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# Supplemental Figure S1

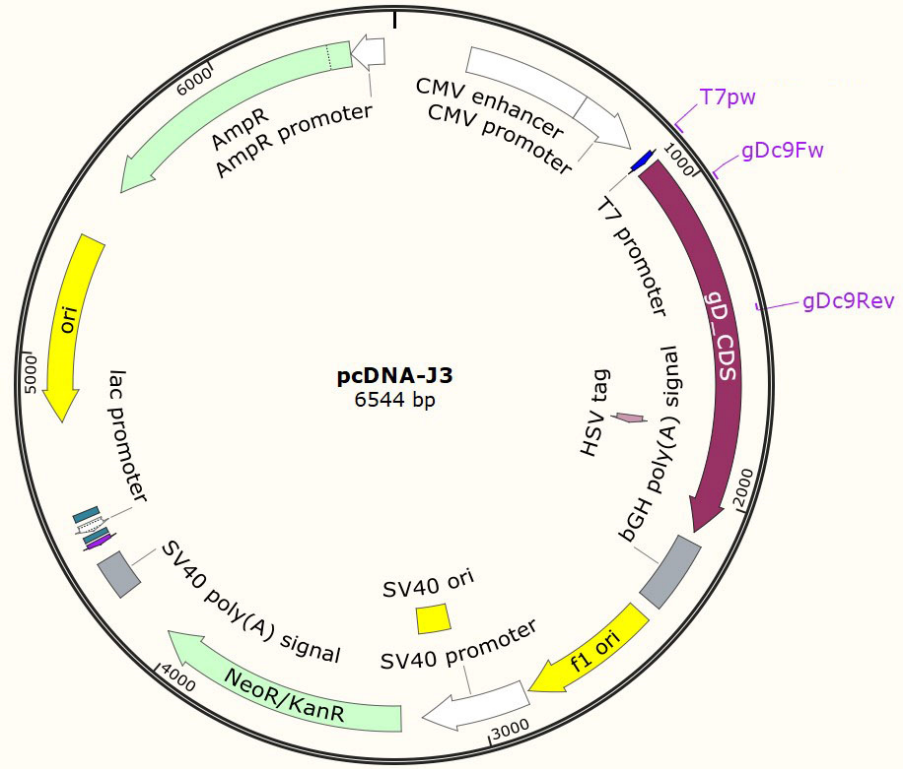
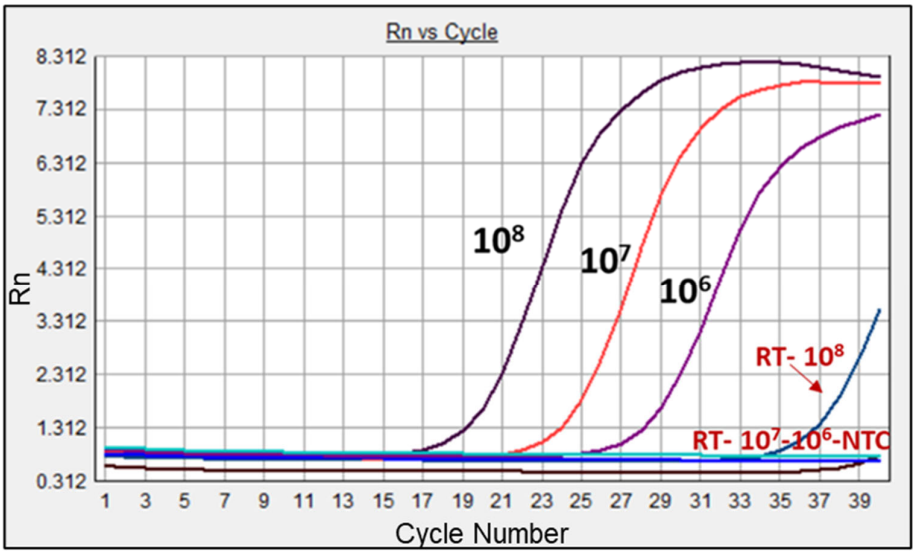



**Figure S1. The pcDNA-J3 expression vector.** Schematic drawing of pcDNA-J3 expression vector contained in I143-J3 cells. The coding DNA sequence (CDS) of HSV-1 US6 gene in pcDNA 3.1 expression vector, corresponding to GenBank accession number L09242.1, is depicted as the dark red region gD-CDS inserted downstream of T7 promoter region (small blue arrow). Position and orientation of the primers used is represented by the violet lines (see Table 1 for amplicon sizes). The map is drawn with SnapGene® software (from Insightful Science; available at snapgene.com).

# Supplemental Figure S2

(a)




(b)

| gD-RNA-synt (number of molecules used for RT reaction) | RT+ | | RT- | | | |
|---|---|---|---|---|---|---|
| | CT average | *St.dev ±* | $Ct_{v1}$ | $Ct_{v2}$ | $Ct_{v3}$ | $Ct_{v4}$ |
| $10^8$ | 17.22 | 0.04 | 38.4 | Undet | 34.4 | Undet |
| $10^7$ | 21.65 | 0.05 | Undet | Undet | Undet | Undet |
| $10^6$ | 26.02 | 0.04 | Undet | Undet | Undet | Undet |

**Figure S2. Optimization of the amounts of gD-RNA-synt to utilize as a template in the RT-qPCR assay.** Real-time PCR analysis of cDNAs produced using three ten-fold dilutions (from $10^8$ to $10^6$ molecules diluited in RNase-water) of gD-RNA-synt as template and $10^{-2}$ U HIV-RT in RT reaction. (a) Amplification curves of one representative experiment where samples containing complete reaction mixtures (RT+, black text) and corresponding negative controls (RT-, red text) are depicted. Each RT- sample contained the same amount of RNA template used in RT+ samples. (b) Ct values of RT+ samples are reported as mean threshold cycle ± standard deviation from four replicates, while Ct values of RT- samples from four replicates are singularly reported. As shown, occasionally amplification of RT- sample corrisponding to $10^8$ molecules of gD-RNA-synt template was detected at high Ct values.

# Supplemental Table S1

**Table S1**. Comparison between the CT values obtained in the same experiment using total RNA or gD-RNA-synt as a template. Reaction conditions: 1X RT-Buffer, 0.2 mM dNTP mix, 0.5 µM gD-reverse primer, 0.025 U HIV-RT, 1 h at 37°C + 5 min at 90°C.

| RNA template | | |
|---|---|---|
| | **CT average** | ***St.dev ±*** |
| **Total RNA (150 ng)** | 25.09 | 0.98 |
| **gD-RNA-synt (10 ng)** | 3.20 | 0.10 |

# Supplemental Table S2

**Table S2**. Comparison between the CT values obtained in the same experiment using fixed amounts of total RNA or gD-RNA-synt as a template and variable amounts of HIV RT. Reaction conditions: 1X RT-Buffer, 0.2 mM dNTP mix, 0.5 µM gD-reverse primer, 1 h at 37°C + 5 min at 90°C.

| RNA template | HIV-RT [U] | RT+ | | RT- | | |
|---|---|---|---|---|---|---|
| | | Average | *St.dev ±* | $Ct_{v1}$ | $Ct_{v2}$ | $Ct_{v3}$ |
| Total RNA (150 ng) | $2.5 \times 10^{-2}$ | 24.71 | 0.12 | Undet | Undet | 32.24 |
| | $2.5 \times 10^{-3}$ | 26.73 | 0.96 | Undet | 32.80 | Undet |
| | $2.5 \times 10^{-4}$ | Undet | - | Undet | Undet | Undet |
| gD-RNA-synt ($0.3 \times 10^{-3}$ ng) | $2.5 \times 10^{-2}$ | 22.40 | 0.18 | Undet | Undet | Undet |
| | $2.5 \times 10^{-3}$ | 24.26 | 0.04 | Undet | Undet | Undet |
| | $2.5 \times 10^{-4}$ | 30.63 | 0.50 | Undet | Undet | Undet |