Supplemental information

HIV reservoir quantification using
cross-subtype multiplex ddPCR

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Figure S1: 5T-IPDA and CS-IPDA assay designs, Related to Figure 1. Location of the primer/probe binding sites in the HIV genome are shown for the 5T-IPDA Assay1 (green stars) and Assay2 (orange stars). The CS-IPDA shown below uses 3 target regions (purple stars) with differences in the HEX concentrations for the LTG/gag and Env targets as compared to the 5T-IPDA.
| Primers and Probes     | Sequence                          |
|------------------------|-----------------------------------|
| 5’ LTR/gag Forward     | GACTAGCGGAGGGCTAGAAGGAGAGA        |
| 5’ LTR/gag Probe       | AT+G+GGT+GC+GAGA                  |
| 5’ LTR/gag Reverse     | CTAATTTCCSCCDCTTAATAYTGACG        |
| 5’ pol Forward         | WCCYTTARYTCCCCTCARATCACTCT        |
| 5’ pol Probe           | TTG+G+CARC+GA+CC                  |
| 5’ pol Reverse         | TACTGTATCATCTGCTCCTGTRTCTAAKAGAGCYT |
| 3’ pol Forward         | AGAGAYCCWMTTGGAAAGGACCAGCMMAA     |
| 3’ pol Probe           | TG+GAAA+G+GT+GAA+GG              |
| 3’ pol Reverse         | CACTACYTTTATDTCACTRTRCTYGTATTACTACTGC |
| tat Forward            | TTTGCTTYMTRACVAAAGGCTTAGGCATCTC(-C) |
| tat Probe              | A+T+G+GCAG+GAA+GAAG              |
| tat Reverse            | TGAGGAGBCYTCGTCGCTG              |
| env Forward            | TVTTCMTTGGGTCTTGGGAGCAGCAGG      |
| env Probe              | A+G+CA+CKA+T+G+GG                |
| env Reverse            | GCACATGCAGAGACAAATAVYTGCTGGCCTGTACC |

Red letters denote changes made to the original primer and probe sequences
+ = locked nucleic acids, (-C) = removal of the C at this position

Table S1: Adaptation of multiplex ddPCR primers and probes to work across subtypes, Related to STAR Methods
| Target Region | Forward Primer Site | Probe Site | Reverse Primer Site |
|---------------|---------------------|------------|---------------------|
| LTR-gag       | GACTAGGGCTAGAGGAGGA | ATGGCTCGAGA | CGTCAAGTATTAAAGGGGAAAATTAG |
|               | [subtypes B]        |            | [subtypes non-B]    |
| S'pol         | TCCATTACCTCCCTCAATCACTCT | TTGCAACGACC | GAAGCTCTTTAGATACAGGACAGATGATACAGTA |
|               | [subtypes B]        |            | [subtypes non-B]    |
| env           | TGTTCTTGGTGCAGCAGGG | GCCACTATGGG | GTTACAGGCCAGAACATTATTATTCTGCTATAGGTC |
|               | [subtypes B]        |            | [subtypes non-B]    |

**Figure S2: Diversity in the CS-IPDA primer binding sites, Related to Figure 2.** Logo plots show diversity in HIV sequences in the LANL HIV database across the CS-IPDA primer and probe binding sites in subtype B (top) and all non-B subtypes (below) in each of the 3 target regions as shown on the left axis. Black circles below the non-B subtype logo plots show sites where degenerate bases are used in the primers/probes with the specific degenerate base noted below.
Figure S3. Accuracy and limit of detection of the cross-subtype IPDA, Related to STAR Methods. HIV DNA from serial dilutions of J-Lat cells (which contain 1 copy of HIV per cell) quantified by CS-IPDA versus a real-time qPCR assay targeting an independent region in HIV pol. Each point represents the average of replicates within an independent experiment and the error bars represent the standard deviation of the replicates. Correlation assessed by Pearson’s $R^2$ with 2-sided p-values.
|               | Subtype B proviral sequences | Subtype C proviral sequences |
|---------------|-----------------------------|-----------------------------|
|               | Defective by CS-IPDA | Intact by CS-IPDA |
| Defective by ProSeq-IT | 901 (84.1%) | 103 (9.6%) |
| Intact by ProSeq-IT    | 1 (0.1%)   | 66 (6.2%)  |
| Defective by ProSeq-IT | 301 (59.3%) | 79 (15.5%) |
| Intact by ProSeq-IT    | 1 (0.2%)   | 127 (25%)  |

Table S2: In-silico analysis of full-length HIV proviral DNA sequences, Related to Figure 2