Abstract #2257

High-throughput Analysis of Full-Length Proviral HIV-1 Genomes from PBMCs

Hiromi Imamichi[1], Yan Guo[2], Colleen Ludka[2], Ellen E. Paxinos[2], H. Clifford Lane[1], Da Wei Huang[3], Richard A. Lempicki[3], and Michael P.S. Brown[2]
[1]NIAID, NIH, DHHS, Bethesda, MD; [2]Pacific Biosciences, Menlo Park, CA; [3]Leidos Biomedical Research, Inc., Frederick, MD

Background

HIV-1 proviruses in peripheral blood mononuclear cells (PBMCs) are felt to be an important reservoir of HIV-1 infection. However, accurate study of this pool is burdened by difficulties encountered in sequencing a full-length proviral genome, typically accomplished by assembling overlapping pieces and imputing the full genome.

Methods

- Cryopreserved PBMCs collected from a total of nine HIV+ patients from 1995-2001 were used.
- High molecular weight DNA was extracted using Puregene DNA Isolation Kit (Qiagen).
- Genomic DNA was subjected to limiting dilution prior to amplification with a near full-length outer PCR followed by nested amplification with an inner PCR (Fig.1).
- The PCR was performed with KAPA HiFi HotStart (KAPA Biosystems) with the initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 98°C for 20s, annealing at 67°C for 15s, and extension at 72°C for 5 min 20s, with the final extension at 72°C for 5 min.
- The PCR products were purified with the BluePippin system (Sage Science) using the 0.75%DF Marker S1 high-pass 4-10kb vs2 cassette definition with BP-Start: 6700 and BP-end: 10000, followed by purification with 1.0X AMPure XP beads (Beckman Coulter).
- Single molecules were sequenced as near-full-length amplicons directly from PCR products without shearing on a PacBio® RS II instrument using commercially available chemistries and protocols for SMRT® Sequencing (P4/C2, 180 min movies).
- Quality of the genomes was validated by clonal positive controls and synthetic mixtures.
- The 1.6-kb gag-pol sequences of plasma virus were obtained by the Sanger method described elsewhere (Imamichi et al., JID, 2001;183:36-50).

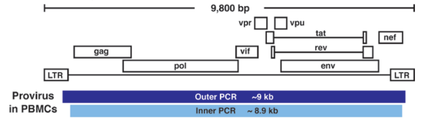


Figure 1. Analysis of HIV-1 provirus (near full-length) and plasma-derived virus (the 1.6-kb gag-pol region).

SMRT Sequencing Methods

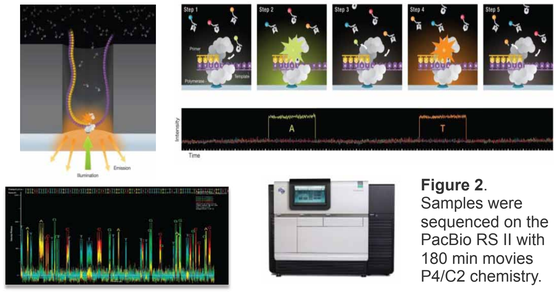
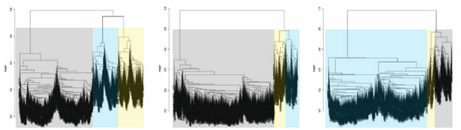


Figure 2. Samples were sequenced on the PacBio RS II with 180 min movies P4/C2 chemistry.

Validation of SMRT Sequencing Results



EXP:	60%20%20%	80%10%10%	15%80%5%
OBS:	59%21%20%	81%10%9%	15%80%5%

Figure 3. Synthetic mixes of three HIV clones: DH12, NL4-3, and AD8 (8.9 kb) separated exactly with correct genomes.

Sequencing of 8.9 kb HIV-1 Proviruses by PacBio

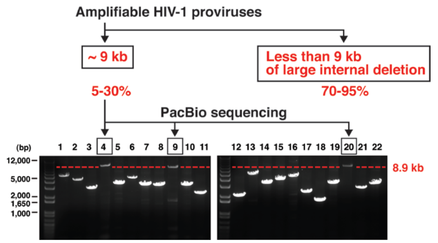
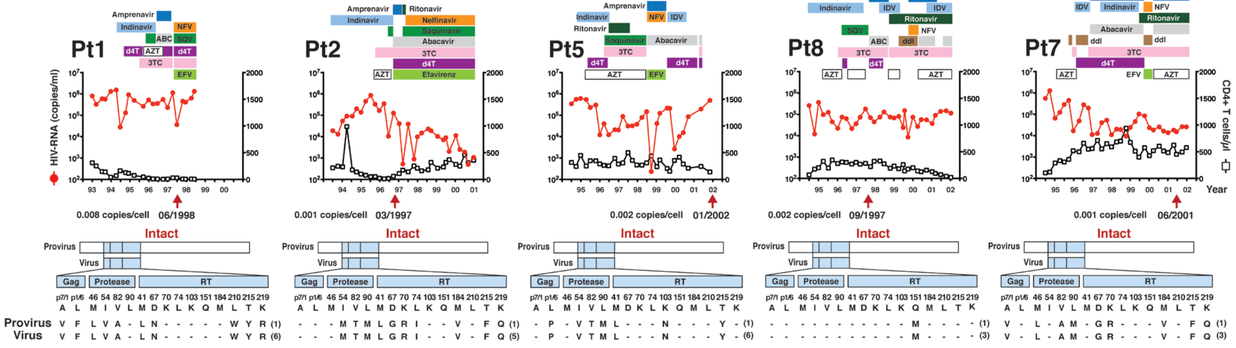


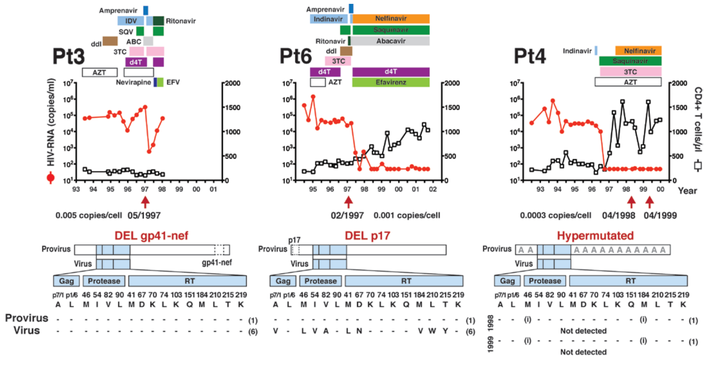
Figure 4. A representative agarose gel analysis of single genome amplicons derived from Pt 9. Approx. 5-30% of amplifiable proviruses were found to be nearly full length.

Sequences of near full-length HIV-1 Proviruses Derived from 9 HIV+ Patients

(1) Intact proviruses



(2) Defective Proviruses



(3) Intact and Defective Proviruses

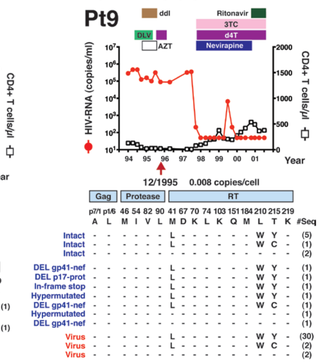


Figure 5. Sequences (1.6-kb encompassing part of the gag-pol region) of provirus and plasma virus from the corresponding time points were compared. Nearly full-length provirus sequences were obtained by the PacBio SMRT Sequencing method; Plasma virus sequences were obtained by the Sanger method. Amino acid positions associated with drug resistance (in the gag cleavage site, protease and a part of reverse transcriptase) are shown in the figure.

Conclusions

- It is possible to amplify near full-length HIV-1 proviruses using the technique described in this study.
- Approximately 5-30% of amplifiable proviruses using this technique were found to be nearly full length.
- Of 24 nearly full-length proviruses obtained in this study, 11 were predicted to be replication-incompetent.
- Every time we identified a competent form of proviruses by the full-length sequencing, we were able to identify that virus in plasma.

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