Efficient amplification of HIV half-genomes from tissue DNA

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The Polymerase Chain Reaction (PCR) amplification of human immunodeficiency virus (HIV) sequences directly from patient samples provides data which shapes our understanding and response to the AIDS pandemic. However, the majority of the sequences obtained to date have resulted from short (< 400 bp) PCR fragments (1, 2, 3), which limits the utility of these data for evolutionary and virologic studies. The amplification of large lentivirus fragments (≥ 4.5 kb) corresponding to one-half of the virus genome, was reported first from an infected cell culture with a high virus load (4), and second from 1 μg of AIDS patient DNA (5). While somewhat successful, the previous methodologies encountered significant drawbacks: tissue culture propagation of virus skews the composition of virus populations (2, 5, 6), co-amplification of multiple templates creates uncertainties with regard to recombination during PCR and bacterial cloning, amplification bias, and Taq polymerase fidelity (1, 5, 7), and amplification of products of the desired size was not always achieved from aliquots of the same patient sample (5).

The method described permits the routine amplification of large HIV fragments directly from patient samples, obviating the need for virus culture prior to amplification, and facilitates functional studies of virus populations present within HIV infected patients. While sensitive amplification of HIV sequences from plasmid templates is readily achieved (Figure A), large fragment amplifications from patient samples often fail, yielding instead short amplified products or smears (Figure B, and ref. 5, unpublished observations). Such reactions often are not limited by the number of provirus targets (approx. 1/1000 cells) (1), but rather seem to fail due to the presence of internally deleted viral DNAs which competitively inhibit the amplification of full length products, and which may exhibit > 1,000 fold preferential amplification relative to 4.5 kb fragments in nested PCR (Figure B). Removal of the shorter molecules by dilution of genomic DNA prior to PCR permits routine amplification of HIV fragments exceeding 4.5 kb in length from as little as a single template molecule (Figure B). Blot hybridization of the gels in Figures A and B yielded the same endpoints for successful amplification, further supporting the claim that single molecule amplification was achieved (data not shown). This approach has been employed in our laboratory for the amplificafion of 5 kb fragments from single proviruses present in HIV and simian immunodeficiency virus infected samples. Such half-genome length fragments have been used to construct replication competent provirus chimeras, and as templates for direct sequencing of viral DNA (unpublished results).

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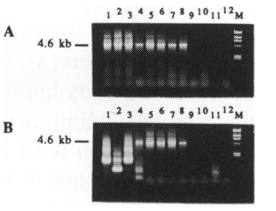

Figure. **A)** 4.6 kb amplification of HIV plasmid PNL4–3 (7). Lanes 1–10 contain 2nd round PCR products amplified from approx. 125, 62, 32, 16, 8, 4, 2, 1, 0.5, 0.25 plasmid molecules, 1 μg of uninfected human DNA (lane 11), and reagents only (lane 12), amplified in 25 μl of PCR reaction mix (10 mM Tris–HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 200 μM each dNTP, 1% DMSO, 10 μg/ml uninfected human DNA, 100 nM primers KKpol-1b and Td4n (5), and 0.6 units Taq Polymerase (Perkin Elmer/Cetus). Reactions were overlaid with 40 μl of mineral oil, and subjected to 35 cycles of amplification (94°C 0.75 min., 55°C 1 min., and 72°C 4 min) followed by 72°C for 10 min. (1st round). Two μl from each reaction was transferred to fresh tubes containing PCR reaction mix and 100 nM primers KKpol3d and KKR4b (5), then reamplified for an additional 35 cycles under the same conditions (2nd round). Ten μl of each reaction was run on a 1% agarose gel, and stained with EtBr. Lane M contains lambda DNA (HindIII digest). **B)** 4.6 kb amplification of HIV-1 from AIDS patient DNA. Lanes 1–10 contain 2nd round PCR products from serial 2-fold dilutions of AIDS patient R.P.DNA, 1,000 ng, 500 ng, 250 ng, 125 ng, 62 ng, 31 ng, 15 ng, 8 ng, 4 ng, 2 ng, 1 μg of uninfected human DNA (lane 11), and reagents only, (lane 12) respectively. Reaction conditions were the same as in A.

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