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10. The pATH13-T plasmid was constructed by means of the pATH 3 expression vector, the *urf13-T* gene of *cms-T* mitochondria, and two complementary synthetic oligonucleotides. Digestion of the clone containing *urf13-T* with Hind III, followed by a partial digestion with Bcl I gave a fragment that included the entire *urf13-T* gene except for the first two nucleotides of the ATG initiation codon. The pATH 3 plasmid was linearized by cleavage of the Eco RI and Hind III sites located within the polylinker cloning region of the vector. Two complementary oligonucleotides corresponding to the sequences 5'-AATTCTGGAGGAAAAAATTAT-3' (top strand) and 5'-GATCATAATTTTTTCCTCCAG-3' (bottom strand) were annealed to yield a fragment with Eco RI and Bcl I sticky ends. The linear pATH 3 vector and the Bcl I–Hind III fragment containing *urf13-T* were then joined by ligation. The oligonucleotides were constructed to provide a prokaryotic ribosome binding site and to reconstitute the initiator ATG codon of *urf13-T*. Cloning procedures were as described [T. Maniatis, E. F. Fritsch, J. Sambrook, in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)]. The structure of the pATH 3 expression vector and conditions of transcriptional induction of the *trp* promoter were according to K. R. Spindler, D. S. E. Rosser and A. J. Berk, [*J. Virol.* 49, 132 (1984)] with modifications described by T. J. Koerner (personal communication). Optimal expression of the pATH13-T vector was obtained in an M9 medium containing casamino acids minus glucose and thiamine. Although the pATH 3 expression vector was designed to enable synthesis of *trpE* fusion proteins, the pATH13-T plasmid was constructed so that the *urf13-T* sequence was out of frame with the *trpE* reading frame allowing production of an unfused 13-kD protein.
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13. A Bcl I–Bgl II fragment [positions 1248 to 1723 according to Dewey *et al.* (6)] containing all of the *urf13-T* reading frame except for the first 11 codons was ligated into the Bam HI site of the expression vector pJG200. The resulting construct (pJG13-T) encoded all of the *urf13-T* reading frame except codons 2 through 11. The pJG200 vector and conditions for induction of transcription are described by J. Germino, J. Charbonneau, T. Vanaman, and D. Bastia [*Proc. Natl. Acad. Sci. U.S.A* 80, 6848 (1983)] and J. Germino and D. Bastia [*ibid.* 81, 4692 (1984)].
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18. We are grateful to H. W. Knoche for his generous gift of BmT-toxin, the E. I. DuPont de Nemours & Co. for the methomyl, T. J. Koerner for the pATH 3 expression vector, and D. Bastia for the pJG200 vector. In addition, we thank H. C. Griffin for excellent technical assistance. Supported by grants from Agrigenetics Corporation and the National Science Foundation. R.E.D. is supported by a North Carolina Biotechnology fellowship. Paper No. 11249 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601.

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DNA Amplification for Direct Detection of HIV-1 in DNA of Peripheral Blood Mononuclear Cells

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By means of a selective DNA amplification technique called polymerase chain reaction, proviral sequences of the human immunodeficiency virus (HIV-1) were identified directly in DNA isolated from peripheral blood mononuclear cells (PBMCs) of persons seropositive but not in DNA isolated from PBMCs of persons seronegative for the virus. Primer pairs from multiple regions of the HIV-1 genome were used to achieve maximum sensitivity of provirus detection. HIV-1 sequences were detected in 100% of DNA specimens from seropositive, homosexual men from whom the virus was isolated by coculture, but in none of the DNA specimens from a control group of seronegative, virus culture–negative persons. However, HIV-1 sequences were detected in 64% of DNA specimens from seropositive, virus culture–negative homosexual men. This method of DNA amplification made it possible to obtain results within 3 days, whereas virus isolation takes up to 3 to 4 weeks. The method may therefore be used to complement or replace virus isolation as a routine means of determining HIV-1 infection.

ALTHOUGH SEROLOGIC ASSAYS IDENTIFY persons with prior exposure to human immunodeficiency virus (HIV-1), they do not specifically determine current infection; this requires isolating the virus from an HIV-1–seropositive person. HIV-1 isolation involves prolonged cocultivation of peripheral blood mononuclear cells (PBMCs) with phytohemagglutinin (PHA)-stimulated lymphocytes from an uninfected donor or with a susceptible uninfected indicator cell line (*1*). The procedure takes up to 3 to 4 weeks and lacks sensitivity in that viruses cannot be consistently isolated from persons with documented infections (*2–4*).

The objective of this study was to directly determine the presence of HIV-1 genetic information in the DNA from PBMCs of a patient and to correlate this with the ability to isolate virus from the same patient. The number of peripheral blood lymphocytes expressing viral RNA, as detected by in situ hybridization (*5*), in an infected person is less than 1 in 10,000 cells; therefore, we used a DNA amplification technique (*6–11*), the polymerase chain reaction (PCR), to amplify specific regions of HIV-1 proviruses present either as the free episomal form or as the integrated form in patients' chromosomal DNA. The amplified viral DNA could be detected by hybridization of a ^{32}P-labeled DNA probe to a portion of the amplified region; a specific restriction endonuclease was then used to cleave the resultant hybrid to yield an HIV-1 diagnostic fragment (*8*). This PCR technique takes less than 3 days to complete.

We tested three groups of patients (Table 1) for the presence of HIV-1 proviral DNA in their PBMCs. Those in group A ($n = 11$) were randomly selected seropositive and virus culture–positive homosexual men from California (*12*); those in group B ($n = 11$) were randomly selected seropositive and virus culture–negative homosexual men from California (*12*). Lymphocytes and sera from persons in these two groups were collected between April 1984 and July 1985. Persons in group C ($n = 13$) were randomly selected negative controls who were seronegative blood donors from the Atlanta area, and their lymphocytes and sera were collected between January 1985 and July 1985.

HIV-1 proviral sequences present in the PBMCs of the persons in groups A through C were amplified by means of primer pairs

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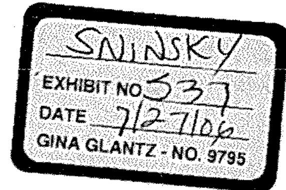

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representing the long terminal repeat (LTR) (SK 29 and SK 30), *gag* (SK 38 and SK 39), and *env* (SK 68 and SK 69 and CO 1 and CO 2) regions of the HIV-1 genome (Table 2). Since HIV-1 shows marked genomic heterogeneity (*13–17*), primer pairs and probes were derived from highly conserved regions of the viral genome (*8*).

For the results with the single primer pair SK 68 and SK 69 (SK 68/69), we used 1 μg of cellular DNA. This DNA was amplified for 35 cycles, hybridized with the ^{32}P-labeled probe, and digested with Hae III (Fig. 1). The appearance of a diagnostic fragment 10 bases in length in the autoradiogram (30% polyacrylamide gel) indicated the presence of HIV-1 proviral sequences in PBMCs. All DNA samples (11 out of 11) from the persons in group A generated the 10-base oligomer, whereas none of the DNA samples from the persons in group C produced the diagnostic oligomer. Of particular interest was the identification of HIV-1 sequences in 5 (46%) of 11 DNA samples from the persons in group B. DNA samples 62, 67, and 70 showed faint oligomer bands, but these were approximately 1/100 of the intensity of the oligomer bands in groups A and B. In addition, similar faint bands were also observed in negative control DNA samples 40 and 49; therefore, 62, 67, and 70 were considered negative.

The same DNA samples were further analyzed with three other primer pairs and probes to minimize problems that might arise from sequence variation in any particular genomic region. Such variation would result in (i) inefficient primer or probe binding, (ii) elimination of specific restriction endonuclease sites, or (iii) both of these problems. As shown in Table 1, the use of all four primer pairs revealed HIV-1 DNA in all of the seropositive, virus culture–positive samples, 7 (64%) of 11 of the seropositive, virus culture–negative samples, and 0 of 13 of the seronegative, virus culture–negative samples. Compared with the single primer pair (SK 68/69), the use of multiple primers enabled us to identify two

Table 1. Correlation of results obtained with the PCR technique by virus culture in seropositive and seronegative persons (*21*). For group C, seronegative and virus culture–negative persons, all samples (40 to 51 and 63) proved to be negative with all four primer pairs.

Samples	Clinical status*	Primer pairs			
		LTR SK29/30	*gag* SK38/39	*env* SK68/69	*env* CO1/2
	Group A: Seropositive and virus culture–positive				
28	Asymptomatic	+++†	+++	+++	++
30	Asymptomatic	+++	+++	+++	++
31	Asymptomatic	+++	+++	+++	++
32	LAS	+++	+++	+++	−
33	LAS	+++	+++	+++	−
34	ARC	+	++	+	+
35	Asymptomatic	+++	+++	+++	+
36	Asymptomatic	+++	+++	+++	++
37	LAS	+++	+++	+++	+
38	ARC	+++	+++	+++	+
39	Hemat. ab.	++	+	+	+
	Group B: Seropositive and virus culture–negative				
60	Asymptomatic	++	++	++	−
61	LAS	++	++	−	+
62	Asymptomatic	−	−	−	−
64	Asymptomatic	−	−	−	−
65	Asymptomatic	−	−	−	++
66	Asymptomatic	−	−	++	−
67	Asymptomatic	−	−	−	−
68	Asymptomatic	++	−	++	−
69	Asymptomatic	−	−	++	−
70	Asymptomatic	−	−	−	−
71	Asymptomatic	−	−	++	++

*LAS, lymphadenopathy syndrome; ARC, AIDS-related complex; hemat. ab., hematologic abnormality (*23*). †Labels +++, ++, +, and − denote high-level, intermediate-level, low-level, and negative, respectively, for the relative intensities of the diagnostic band observed in the autoradiograms.

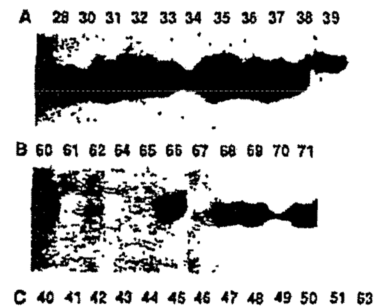

Fig. 1. (**A** to **C**) Representative DNA amplification analysis of peripheral blood lymphocyte DNA from HIV-1–seropositive and seronegative persons (see Table 1). DNA samples were amplified for 35 rounds with the primer pair SK68/69 (Table 2) representing a conserved gp41 region, restricted with BstN I and fractionated in a 30% polyacrylamide gel. The detailed experimental procedures are described in (*21*).

Table 2. Sequences of oligonucleotide primer pairs and probes and their locations in the HIV-1 genome.

Primer or probe		Sequence (5′–3′)	Location in HIV-1*
SK 29	Primer	ACTAGGGAACCCACTGCT	LTR 501–518
SK 30	Primer	GGTCTGAGGGATCTCTA	LTR 589–605
SK 31	Probe	ACCAGAGTCACACAACAGACGGGCACACACTACT	LTR 552–585
SK 38	Primer	ATAATCCACCTATCCCAGTAGGAGAAAT	*gag* 1551–1578
SK 39	Primer	TTTGGTCCTTGTCTTATGTCCAGAATGC	*gag* 1638–1665
SK 19	Probe	ATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCTAC	*gag* 1595–1635
SK 68	Primer	AGCAGCAGGAAGCACTATGG	*env* 7801–7820
SK 69	Primer	CCAGACTGTGAGTTGCAACAG	*env* 7922–7942
SK 70	Probe	ACGGTACAGGCCAGACAATTATTGTCTGGTATAGT	*env* 7841–7875
CO 1	Primer	ACAATTATTGTCTGGTATAG	*env* 7855–7874
CO 2	Primer	AGGTATCTTTCCACAGCCAG	*env* 7970–7989
CO 3	Probe	TGAGTTGCAACAGATGCTGTTGCGCCTCAATAGCCCTCAG	*env* 7895–7934

*HIV-1 isolate ARV-2 (*24*), GenBank accession number KO2007.

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additional (61 and 65) HIV-1–positive persons from the seropositive, virus culture–negative group.

A positive reaction does not necessarily indicate that the HIV-1 proviruses detected could replicate in the patient's lymphocytes or in tissue culture. This may have been the case with the seven PCR-positive persons in group B (Table 1). The inability to recover virus from these persons could have been due to defective proviruses. The presence of defective integrated proviruses of HIV-1 was reported previously (*18*). Defective provirus would be detected by the PCR technique provided the region targeted for amplification was preserved.

There are three possible explanations for the identification of persons who were seropositive but both virus culture–negative and PCR-negative. First, these persons may have contained an insufficient number of provirus copies (that is, very few infected lymphocytes) to be directly detected by the PCR technique described here. It might be possible to increase the sensitivity of detection by using additional cycles or by using RNA instead of chromosomal DNA as the original template. The viral RNA would be converted to complementary DNA with reverse transcriptase and then subjected to DNA amplification. The increased sensitivity would result from the higher number of virus-specific RNA molecules than proviral DNA in infected cells (*5*). However, the use of RNA as the initial template and subsequent amplification would only be possible if the HIV-1 provirus in an infected PBMC was biologically active.

Second, these persons may have been infected with HIV-1 containing genetic variations or deletions in the regions targeted for amplification. This appears unlikely, since the use of four primer pairs and probes representing conserved sequences from three different regions (LTR, *gag*, and *env*) of the viral genome failed to detect HIV-1 proviruses in these persons.

Third, these persons may not have harbored HIV-1 proviruses. The lack of HIV-1 proviral sequences that react with our probes in the PBMCs of these people could have resulted from their originally having been exposed to noninfectious HIV-1 antigens or from their successful elimination of the virus from their PBMCs. Interestingly, a high proportion of sex partners of seropositive persons who have had unprotected sex appear to have remained seronegative (*19*). The PCR technique described here may prove useful in determining whether the seropositive persons have detectable levels of HIV-1 proviruses and whether their sex partners were subsequently infected even though the partners remain seronegative. An analogous situation existed with infants born to seropositive mothers (*20*). Some of these infants lost their maternal HIV-1 antibodies and remained asymptomatic, whereas the others developed their own HIV-1 antibodies and acquired immune deficiency syndrome (AIDS) or AIDS-related complex (ARC). The results presented in this report demonstrate the potential utility of the PCR technique in complementing or replacing virus isolation as a routine means of determining the presence of HIV-1.

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21. Sera and PBMCs were collected and processed as previously described (*3*, *4*) as was the isolation of DNA from PBMCs (*22*). The HIV-1 antibody status of patients was initially determined by an enzyme-linked immunosorbent assay (ELISA) and subsequently confirmed by Western blot testing (*3*). The presence of HIV-1 in PBMCs was monitored by the appearance of reverse transcriptase activity in PBMCs cocultivated with PHA-stimulated lymphocytes from healthy seronegative donors (*1*). The PCR reaction mixture contained 1 μg of PBMC DNA, 100 pmol each of primers (Table 2), 10 m*M* each of four deoxyribonucleoside triphosphates, 10 m*M* tris-HCl, *p*H 7.5, 50 m*M* NaCl, 10 m*M* $MgCl_2$, and 0.6 unit of thermoresistant DNA polymerase of *Thermus aquaticus*. The amplification was carried out as described by R. Saiki *et al.* (*Science*, in press) with an automated temperature device (Cetus). After the amplification steps, one-tenth of the reaction mixture was removed, the NaCl concentration was adjusted to 0.15*M*, and the mixture was subjected to hybridization with a ^{32}P-labeled adenosine triphosphate end-labeled probe (Table 2) at 56°C for an hour. Hybridization of the labeled probe with the amplified HIV viral sequence yielded a double-stranded DNA form that was sensitive to selective restriction enzyme digestion (*6*). The restriction enzymes used for primer pairs SK29/30, SK38/39, SK68/69, and CO1/2 were Hinf I, BstN I, Hae III, and Hha I, respectively. Restricted samples were analyzed on a 30% polyacrylamide gel. Autoradiograms of the portion of the gel with the diagnostic labeled fragment were obtained by exposure of Kodak XAR film at −70°C for 3 to 4 hours in the presence of an intensifying screen.
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