Degenerate and specific PCR assays for the detection of bovine leukaemia virus and primate T cell leukaemia/lymphoma virus pol DNA and RNA: phylogenetic comparisons of amplified sequences from cattle and primates from around the world

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Degenerate and specific PCR assays were developed for bovine leukaemia virus (BLV) and/or primate T cell leukaemia/lymphoma viruses (PTLV). The degenerate assays detected all major variants of the BLV/PTLV genus at a sensitivity of 10–100 copies of input DNA; the specific systems detected 1–10 copies of input target. Sensitivity was 100% in specific DNA–PCR assays done on peripheral blood from seropositive BLV-infected cattle and HTLV-I- or HTLV-II-infected humans, and 62% in RNA/DNA–PCR assays on sera from BLV seropositive cattle. The pol fragments from 21 different BLV strains, isolated from cattle in North and Central America, were cloned and sequenced, and compared to other published BLV and PTLV pol sequences. BLV and PTLV sequences differed by 42%. Sequence divergence was up to 6% among the BLV strains, and up to 36% among the PTLV strains (with PTLV-I and PTLV-II differing among themselves by 15% and 8%, respectively). Some cows were infected with several BLV strains. Among retroviruses, BLV and PTLV sequences formed a distinct clade. The data support the interpretation that BLV and PTLV evolved from a common ancestor many millennia ago, and some considerable time before the PTLV-I and PTLV-II strains diverged from each other. The dissemination of the BLV strains studied probably resulted from the export of European cattle throughout the world over the last 500 years. The relatively similar mutation rates of BLV and PTLV, after their various points of divergence, suggest that there could be a much wider genetic range of BLV than has currently been defined.

Introduction

Bovine leukaemia virus (BLV) is an infectious agent of cattle that can cause lymphomas and benign disorders which, directly or indirectly, have a financial impact on the cattle industry (Ferrer, 1980; Brenner et al., 1989; Burny et al., 1990). It is estimated that more than 10% of dairy cattle in the United States are infected with BLV. BLV, together with primate T cell leukaemia/lymphoma virus (PTLV), form a separate genus of retroviruses that exhibit in vivo lymphotropism and are characterized by the transforming property of a unique virus regulatory protein, Tax, which can transactivate both viral and cellular genes (Fig. 1) (for review see Poiesz, 1995). Because of the development of sensitive and subtype-specific PTLV PCR assays, which allow for easy cloning and sequencing, much is known about the dissemination and phylogeny of PTLV, whereas only limited data exist for BLV.

We have reported that a region of the pol gene which encodes for part of the integrase protein in all retroviruses could prove useful in PCR-based epidemiological and phylogenetic studies. Primers can be made from relatively conserved sequences that flank a small (~140 bp) segment and whose
 sequencing eight BLV seropositive peripheral blood samples tested. The Poisson distribution, and detected BLV sequences in all DNA method was sensitive down to one copy, according to target for the specific detection of BLV DNA and RNA. The flanked by the PTLV-generic primer-pair SK 110 pol here PCR-based methods using the region of the retroviruses. All branches have highly significant bootstrap values (> 80%) with most being 100%. The seven universally recognized major groups or genera of exogenous retroviruses are indicated. As can be seen, there are five ancient points of divergence from a common virus ancestor occurring after separation into the seven genera. Species infecting humans can be found in the lentivirus, spumavirus and PTLV/BLV genera.

Fig. 1. An unrooted neighbour-joining distance tree showing the relationships of entire pol gene protein sequences of different exogenous retroviruses. All branches have highly significant bootstrap values (> 80%) with most being 100%. The seven universally recognized major groups or genera of exogenous retroviruses are indicated. As can be seen, there are five ancient points of divergence from a common virus ancestor with two branches characterized by the presence or absence of dUTPase. It is presumed that the acquisition of regulatory genes (e.g. tax, rex or tat, rev) by some of the genera represent independent evolutionary events occurring after separation into the seven genera. Species infecting humans can be found in the lentivirus, spumavirus and PTLV/BLV genera.

The degree of diversity correlates linearly with that of both LTR sequences and the complete retrovirid genomes (Dube et al., 1993, 1994, 1995). We have already developed rapid and sensitive, generic and specific, isotopic and non-isotopic DNA/RNA–PCR assays within this region for the PTLV subsets, human T cell leukaemia/lymphoma viruses I and II (HTLV-I and HTLV-II) and simian T cell leukaemia virus I (STLV-I) (Kwok et al., 1988; Ehrlich et al., 1989; Saksera et al., 1994; Dyster et al., 1994). Furthermore, we have developed an extensive sequence database of this region, and comparative analyses of these 140 bp among different retroviruses have proven highly accurate in predicting statistically significant phylogenetic relationships based on substantially greater sequence data (Dube et al., 1993, 1994, 1995; Ferrer et al., 1996).

We have previously published PCR assays for BLV (Sherman et al., 1992 a); however, they were not directed at the above mentioned informative region of pol. Hence, we describe herein PCR-based methods using the region of the pol gene flanked by the PTLV-generic primer-pair SK 110/111 as the target for the specific detection of BLV DNA and RNA. The DNA method was sensitive down to one copy, according to the Poisson distribution, and detected BLV sequences in all eight BLV seropositive peripheral blood samples tested. The sequenced pol DNA from 24 different BLV strains from Japan, Australia, Europe, North America and Central America were compared phylogenetically among themselves, to the PTLV strains and to other exogenous retroviruses. Furthermore, we describe a generic assay utilizing degenerate primers capable of detecting all known members of the BLV/PTLV genus.

Methods

Cattle. Sera and heparinized peripheral blood were obtained from cattle from Costa Rica, Honduras, Venezuela and the United States, including a high-incidence lymphoma herd (BF) maintained at the New Bolton Center, University of Pennsylvania School of Veterinary Medicine (Table 1). The characteristics of the BF herd have been described (Ferrer et al., 1993, 1994, 1995). We have already developed rapid and sensitive, generic and specific, isotopic and non-isotopic DNA/RNA–PCR results in BLV seropositive cattle.

Table 1. RNA or DNA BLV-specific PCR results in BLV seropositive cattle

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* DNA was obtained from PBMC.
† RNA was obtained from sera.
‡ BFP, parental BF herd.
§ BFI, BF herd inbred for lymphoma.
et al., 1974; Abt et al., 1976). It should be noted that over a 12 year period bulls and cows from the BF herd with a diagnosis of lymphoma were included. Samples taken from the parental BF herd are designated BFP and those taken from the inbred cattle are designated BFI.

**Primates.** Heparinized peripheral blood was obtained from 20 each of HTLV-I-positive, HTLV-II-positive, HTLV-I-negative and HTLV-II-negative humans and 10 STLV-I-positive non-human primates.

**Cell lines.** The plasmid pBLV-1 (Deschamps et al., 1981) and the cell lines FLK/BLV (Van der Maaten et al., 1972), UMC-CTCL 11B (Ehrlich et al., 1989a) and MoT (Kalyanaraman et al., 1982) were used as the controls for BLV, HTLV-I and HTLV-II, respectively. The relevant retroviral copy numbers in these cell lines had previously been determined by Southern blot hybridization and confirmed by PCR analyses of serially diluted lysed cells and calculation of the Poisson distribution (Ehrlich et al., 1989a; Sherman et al., 1992a).

**Serological assays.** Cattle were screened for BLV antibodies using radioimmunoassays in which the purified 125I-labelled major core (p25) or envelope (gp51) BLV proteins were used as antigens (McDonald & Ferrer, 1976; Gupta & Ferrer, 1981). Humans and non-human primates were screened for HTLV-I and HTLV-II antibodies using commercial ELISA and Western blot assays (Cellular Products) (Dube et al., 1995).

**Design of primer and probe oligonucleotides.** All primers and probes were synthesized on a DNA synthesizer (Applied Biosystems). The relevant available nucleotide sequences of the pol gene from HTLV-I, STLV-I, HTLV-II (Dube et al., 1995), BLV (Sagata et al., 1985; Coulston et al., 1990; Deschamps et al., 1981) and the retroviruses Moloney murine leukemia virus (MoMLV), simian foamy virus (SFV), visna virus, human immunodeficiency viruses types 1 and 2 (HIV-1 and -2), simian immunodeficiency viruses SIVse and SIVcpz (GAB), bovine immunodeficiency virus (BLV), mouse mammary tumour virus (MMTV), Mason-Pfizer monkey virus (MPMV), Rous sarcoma virus (RSV) and squirrel monkey retrovirus (SMRV) (GenBank accession numbers J02342 and M23385, respectively), were aligned using the GCG software package (Devereux et al., 1984) and MASE (Faulkner & Jurka, 1988). Specific oligonucleotides from the homologous regions of the BLV pol gene, which encompass or are flanked by the 3'-region of the BLV primer-pair SK 110/111 (Dube et al., 1989, 1992), were designed as follows: BLV 110(+), 5'-CCCTACAACCCCACAAGTTCGG 3'; BLV 111(−), 5'-ATGCGTGCCCTCAGTCTCCTT 3'; primer; BLV 115(+), 5'-TCGAGCCCTCTGGACTCACAATGCAGTTAACCT 3'; probe; BLV P1(+), 5'-GCTGCTCAAACTTCTTC-3'; BLV P1(−), 5'-AGCCTCAAACTTCTTC-3'; BLV P1, 5'-AGCCTCAAACTTCTTC-3'.

The BLV/PTLV degenerate oligonucleotides were as follows: BLV/P-PTLV 110(+), 5'-CCCTACAACCCCACAAGTTCGG/AAGCCT/TCTA/GGC3'; primer; BLV/PTLV 111(−), 5'-G/A/CTGTTG/G/TATTGG/C/GCCATGC/TGGTTTC/CTT3'; primer; BLV/PTLV 115(+), 5'-CCCTACAACCCCACAAGTTCGG/AAGCCT/TCTA/GGC3'; primer; BLV/PTLV 111(−), 5'-G/A/CTGTTG/G/TATTGG/C/GCCATGC/TGGTTTC/CTT3'; primer; BLV/PTLV 115(+), 5'-CCCTACAACCCCACAAGTTCGG/AAGCCT/TCTA/GGC3'.

The bases in parentheses represent the various degeneracies that were synthesized to allow for the amplification and detection of either PTLV or BLV pol sequences. All primers also included 5' linker sequences to facilitate subsequent cloning of the amplified DNA, and to label all amplified products with signature sequences (Dube et al., 1993; Abbott et al., 1994). The positive-strand linker sequence 5'-ACAGTACACCTGAGATCTAGA 3' contains a KpnI site and the negative-strand linker sequence 5'-TACCAGCTCGCGAATTCCTAGA 3' contains an SstI site.

**PCR assays.** PBMC were obtained by centrifugation of bovine, human or simian peripheral blood on Ficoll–Hypaque. DNA was obtained by organic extraction and 1 µg was subjected to PCR amplification and detection with PTLV primer pairs and probes as previously described (Dube et al., 1995), the BLV-specific primers BLV 110/111 and probe BLV P1, or the degenerate primers BLV/PTLV 110/111 and probe BLV/PTLV 115. The BLV-specific and BLV/PTLV degenerate DNA–PCR assays were optimized for sensitivity and specificity, resulting in the following conditions: 10 mM Tris–HCl, pH 8.3; 50 mM KCl; 17 µg BSA (Sigma); 3 U Tag DNA polymerase (a kind gift of Roche Molecular Systems); a 50-fold molar excess of anti-Taq antibody TP1; a 5-fold molar excess of anti-Taq antibody TP4 (kindly provided by Johnson & Johnson Clinical Diagnostics Systems) (Sharkey et al., 1994); 2.5 mM MgCl₂, 50 pmol of each primer; 270 µM each of dATP, dGTP, dCTP and dUTP (Pharmacia LKB Biotechnology); and 60 cycles of amplification in a 480 DNA thermocycler (Perkin-Elmer Cetus). Cycle parameters were described previously (Sherman et al., 1992a).

**RT–PCR assay.** RNA was extracted from 500 µl of serum sample by the guanidinium isothiocyanate method, as previously described (Dube et al., 1994). The RNA pellet was resuspended in 24 µl of RNase-free, gene-screen filtered, deionized water. Viral RNA was transcribed to cDNA using MoMLV RT (Gibco BRL). To 12 µl of RNA solution was added 18 µl of reaction mixture (50 mM Tris–HCl, pH 8.3; 75 mM KCl; 10 mM DTT; 3 mM MgCl₂; 600 µM each of dGTP, dATP, dUTP and dCTP; 50 pmol of negative-strand BLV pol primer BLV 111 and 200 U of MoMLV RT). The reaction mixture was incubated at 55 °C for 5 min and then at 42 °C for 20 min. Amplification of the cDNA was accomplished by adding 70 µl of PCR reaction mixture to give the final concentrations given above for the DNA–PCR assay. The cDNA was amplified for 60 cycles. It should be noted that the RNA sample prior to RT–PCR was not treated with DNase so as to maximize the amplification product which may be amplified from both RNA and DNA rather than RNA only. All PCR assays were done by utilizing dUTP rather than dTTP and all samples were ‘pre-sterilized’ by treatment with uracil-N-glycosylase (Longo et al., 1990). All pre- and post-PCR analyses were done by separate personnel and in different buildings in order to avoid false-positives due to carry over contamination by previously amplified DNA. This was further confirmed by retesting the sample with ‘signature’ primer-pairs, which were composed of the non-human, non-viral linker sequences mentioned above (Abbott et al., 1994). All amplified products were subjected to Southern blot analysis with 32P-labelled BLV or BLV/PTLV probes as previously described (Dube et al., 1995).

**Cloning and nucleotide sequencing.** Ligation and cloning of the PCR-amplified DNA into the M13mp18 vector after digestion with KpnI and SstI were done as previously described (Dube et al., 1993). The ligated DNA was used to transfected Escherichia coli KBT052 (Unr+) cells (Kunkel et al., 1987). Positive clones were detected by plaque-hybridization with BLV P1 and the viral DNA was sequenced by the dideoxynucleotide chain-termination method (Sanger et al., 1977).

**Phylogenetic analysis.** Multiple clones (at least three) were sequenced per sample derived from cattle. Sequences were aligned and illustrated using the GCG programPILEUP and MALIGNED 1.4 (Clark, 1991), respectively. These alignments were edited according to known functional properties of the amino acid sequences of cognate proteins encoded by all retroviral pol genes (Johnson et al., 1986). The BLV 110/111 pol DNA sequences were compared to each other using the maximum parsimony programPAUP (Swofford, 1991). The neighbouring joining method, utilizing the maximum-likelihood distances (Felsenstein, 1989), was used to compare new and previously published BLV, HTLV-
I, STLV-I and HTLV-II pol 110/111 DNA sequences (Dube et al., 1995). The entire pol amino acid sequences from representative unique PTLV and BLV strains, and all the PTLV and BLV 110/111 DNA sequences, were compared to homologous sequences from all of the other exogenous retroviruses mentioned above. Dendrograms were constructed to delineate the evolutionary relationships among these different virus groups. Confidence limits on branching patterns in neighbour-joining trees were estimated by analysing 100 bootstrap replicates.

**Results**

Using the cell line FLK/BLV, the plasmid pBLV-1 and purified virions as quantitative controls, both DNA- and RNA-directed BLV-specific PCR assays using the probe BLV P1 were developed. These assays showed a sensitivity of input nucleic acid down to 1 and 10 copies, respectively, according to the Poisson distribution (Fig. 2 and data not shown). Specificity was demonstrated by the lack of detection of BLV sequences in DNA and RNA extracted from BLV seronegative cattle and the lack of cross-reactivity to HTLV-I, HTLV-II and STLV-I sequences. Assays using the probe BLV 115 were just as sensitive but, because of the greater homology of BLV 115 to PTLV sequences, were not as specific (data not shown). Eight out of eight BLV seropositive PBMC samples were positive for BLV DNA and 16 out of 26 (62%) plasma samples were positive for BLV RNA (Table 1). The degenerate BLV/PTLV assay system was not as sensitive as the respective specific assays, but was capable of detecting BLV, HTLV-I, HTLV-II and STLV-I down to an input of 10–100 copies (Fig. 3 and data not shown). All 20 HTLV-I- and HTLV-II-positive and the 10 STLV-I-positive PBMC samples were correctly scored as positive in the PTLV DNA–PCR system, whereas none of the seronegative samples were positive. The STLV-I samples have been previously reported (Saksena et al., 1994).

Twenty amplicons from the BLV-specific PCR amplification of 100 copies of the plasmid pBLV-1, and five amplicons from the amplification of 100 copies of BLV from the cell line FLK/BLV were cloned and sequenced. Each set of clones gave uniform sequences, indicating the high fidelity of the system and the lack of markedly divergent BLV quasi-species formation in vitro. The pBLV-1 sequence was identical to the published sequence and differed from the FLK/BLV sequence by 2–7% (Fig. 4). Twenty BLV pol sequences were obtained from the cloned amplicons derived from cattle samples, and were compared to the pBLV-1, FLK/BLV, and the published iBLV and PBLV-A1 BLV sequences. The various BLV sequences diverge from each other by 0–6·3% (Fig. 4), with the
BLV38475(5) strain from the United States being the most divergent relative to the pBLV-1 sequence. Whereas most of the nucleic acid changes were synonymous, several non-synonymous changes were observed (Fig. 4).

Interestingly, several cattle seemed to be infected with more than one BLV strain (Figs 4 and 5). Whereas the strains from the BFI herd at the University of Pennsylvania, which had been inbred for lymphoma, demonstrated marked conservation of BLV sequences, individual outbred cattle from both Honduras and the United States, including those from the parental BF herd, were infected with multiple strains whose sequences diverged up to 5% among themselves (Figs 4 and 5).

The PTLV strains diverged by up to 36%, with PTLV-I and PTLV-II differing among themselves by 15% and 8%, respectively. There was up to 6% divergence among BLV sequences; however, unlike PTLV sequences, there were insufficient data to group them into statistically significant, geographically distinct, substrains (Figs 5 and 6). However, BLV sequences were significantly separate from PTLV sequences. As anticipated (Fig. 1), BLV strains clustered as a group with a common ancestor to PTLV, and with PTLV forming a genus distinct from all other retroviruses (data not shown, Figs 5 and 6). Of the other exogenous retroviruses compared, RSV and MPMV were the most homologous to the BLV/PTLV sequences. The mean divergence of the PTLV-II strains to the BLV strains (41.8% ± 0.5%) were not significantly different (Students’ t-test). Likewise, the mean divergence of the PTLV strains to RSV (59.8% ± 0.6%) and the mean divergence of the BLV strains to RSV (59.4% ± 1.0%) were also very close but were statistically different (P < 0.02; Students’ t-test).

Discussion

BLV and PTLV form a unique genus of retroviruses which exhibits in vivo lymphotropism and a low level of oncogenesis in their respective hosts. The pathogenesis of the neoplastic diseases associated with these viruses is believed to involve the transactivation of critical host cellular genes by the BLV/PTLV viral transcriptional transactivator protein, Tax. Approximately 10–30% of cattle or primates infected by BLV or PTLV, respectively, ultimately suffer from neoplastic or benign ailments which affect the quality and length of life and, in the case of cattle, agricultural productivity.

Because of their relevance to human health the detection, dissemination and phylogenetic relationships of PTLV have been extensively studied, resulting in modest advances in the prevention, diagnosis and treatment of their associated diseases. It is important to have sensitive and specific assays for BLV as well, because of the potential economic impact of BLV infection. As it is widely believed that a common ancestor to BLV and PTLV was transmitted to human and/or non-human...
primates many millennia ago (presumably from a bovid) it would also seem important to understand the complete global diversity of the BLV/PTLV genus.

In our studies of PTLV, DNA- and RNA-directed PCR assays have proven to be extremely sensitive and specific assays for virus detection. Furthermore, they have allowed for easy cloning and sequencing, and subsequent phylogenetic analysis. A particular region of the PTLV pol gene, flanked by the primers SK 110/111, has proven especially useful for these purposes. Accordingly, as described herein, we were successful in developing sensitive and specific DNA- and RNA-directed PCR assays for the pol region of BLV, which is homologous to SK 110/111 of PTLV. The performance of the BLV DNA–PCR assay was comparable to the PTLV assay. The RNA-directed PCR assay on archival bovine plasma samples was not as sensitive as the DNA-directed PCR assay on bovine PBMC DNA, as we had found previously with PTLV (Dube et al., 1994). The reasons for this discrepancy are unclear but presumably are secondary to: (1) low levels of retroviral expression in BLV/PTLV-infected animals relative to other animal retroviruses; (2) degradation of the stored plasma viral RNA sample; and (3) PCR inhibitors in plasma. It is likely that virus immunoprecipitation techniques, which have proven useful in plasma HIV RNA detection (Zhang et al., 1994), would overcome some of the above obstacles to BLV/PTLV detection.

The phylogenetic data derived herein were as anticipated. Our analyses of BLV pol sequences from domesticated cattle from Europe, Australia, Japan and the Americas revealed a 6% divergence, presumably reflecting the dissemination of European cattle around the world over the last 500 years. This result is very similar to that obtained by others who have compared BLV env sequences (Mamoun et al., 1990). An interesting observation was the fact that several cows were

![Dendrogram](image-url)
Fig. 6. Neighbour-joining distance trees showing the relationship of the pol gene protein sequences flanked by either the BLV 110/111 or SK 110/111 primers of BLV or PTLV, respectively, and the homologous region of RSV which was used as an outgroup. Two visual versions of the same data (a and b) are shown to facilitate the recognition of the phylogenetic relationships and individual strains. The lengths of the branches correlate with the divergence between the isolates. The bootstrap confidence values (100 replicates) between the RSV, BLV, PTLV-I and PTLV-II branches were 100%, whereas those between the major subgroups of PTLV-I (African and Asian/Austronesian) and PTLV-II (A and B) ranged from 63% to 100% (data not shown). The data also suggest BLV strains may represent distinct subgroups, but the bootstrap values are not significant when comparing this limited amount of sequence. A tree generated using MPMV as an outgroup gave a very similar pattern (data not shown).
infected with multiple BLV strains whose divergence ranged from 1.5–5%. It is doubtful whether these differences can be deemed artifactual and secondary to errors during PCR amplification, cloning and sequencing in that no mutations were observed in 20 and five cloned sequences of pBLV-1 and FLK/BLV, respectively. Likewise, given the degree of conservation of the pol sequences in the FLK/BLV cell line and in PTLV-infected humans and cell lines tested to date (Ehrlich et al., 1992; Sherman et al., 1993), it is unlikely that the degree of BLV strain divergence observed in some cattle was the result of quasi-species formation following infection by one strain. Whereas we have found humans co-infected with both HTLV-I and HTLV-II (which diverge from each other by 40%), we have yet to identify humans or non-human primates co-infected with multiple PTLV-I or PTLV-II strains (Ehrlich et al., 1989b, c, 1992; Dube et al., 1993, 1994, 1995; Saksena et al., 1994). These observations may reflect differences in immunologically protection against BLV and PTLV infection in cattle vs primates, respectively, or they may be due to simultaneous inoculation of multiple BLV isolates into a particular cow via veterinary or animal husbandry techniques.

Another interesting observation was the highly conserved BLV sequences found in the BFI herd which had been inbred for the high incidence of B-cell lymphomas. The parental herd, BFP, from which the BFI group was derived, is infected with this same strain but is also infected with multiple other BLV strains (Figs 4 and 5). Whereas PTLV-I infection is associated with a higher incidence of T cell neoplasia than PTLV-II infection, no relationship to sequence variation and leukaemia incidence has been observed among PTLV-I strains (Poiesz, 1995). One can only speculate whether the homogeneity of the BFI BLV isolates represents a founder effect in a group of genetically susceptible cattle or demonstrates an increased transforming capability of a substrain of BLV.

Our data also indicate that, after their points of separation, PTLV-I, PTLV-II and BLV have mutated at relatively similar rates. This observation would suggest that there could be as much divergence among modern BLV strains as there is among PTLV strains. BLV data derived to date from domesticated cattle do not support this hypothesis (Figs. 1, 5 and 6). However, one can presume that more varied BLV strains could be found in other extinct or living animals (most likely, but not necessarily, bovids). The degenerate BLV/PTLV PCR systems described herein should prove useful in the search for such variants.

A comparison of BLV and PTLV 110/111 pol DNA sequences, in which prototype HTLV-I and HTLV-II sequences were used as an outgroup, indicates that PTLV form a phylogenetic group distinct from BLV (Fig. 5), a conclusion consistent with the analysis of the entire pol gene protein sequences of all exogenous retroviruses including BLV and PTLV (Fig. 1). However, when MPMV or RSV 110/111 DNA sequences are used as an outgroup to BLV and PTLV sequences, these patterns are not as clear and BLV sequences tend to segregate together with PTLV sequences. This observation may be artifactual and secondary to errors in comparing the small number of bases flanked by the 110/111 primers or it may be due to an ancient recombination event between BLV and PTLV strains encompassing the 110/111 region of the pol gene. This latter hypothesis has been proposed previously by ourselves and others using different analytical techniques (Doolittle et al., 1989; Dube et al., 1993, 1995). Confirmation of such a recombinational event would require the careful comparative analyses of many dissected full-length and variant PTLV and BLV strains. Again, the BLV and BLV/PTLV PCR systems described herein should allow for the detection of such additional retroviral sequences.

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