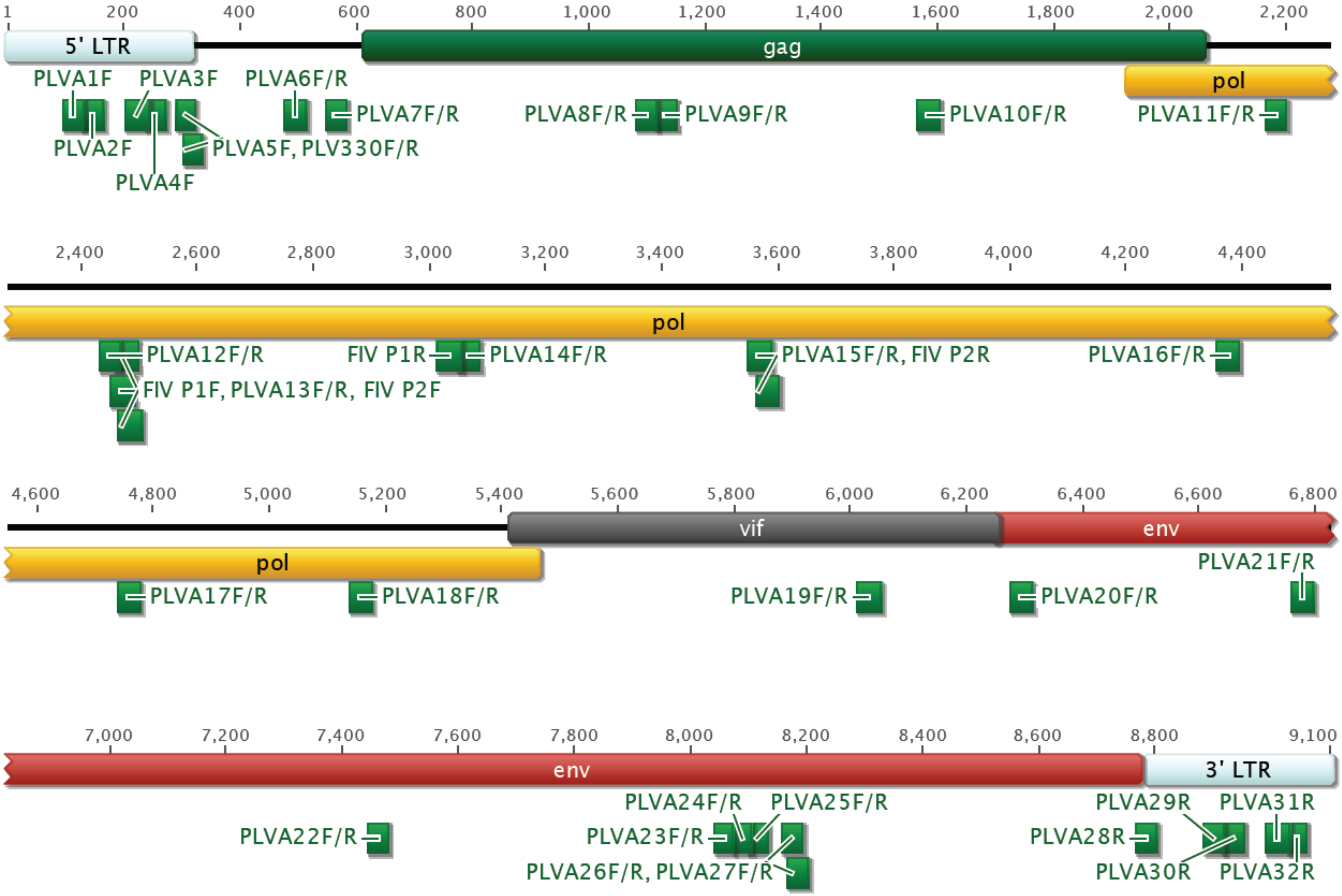

Figure A1a: PLVA and PLVB primer maps showing the location of the primers (small green boxes) utilized in this study. Each figure is annotated with the location of the 5'- and 3'- long terminal repeats (LTR) and open reading frames (*gag, pol, vif, OrfA* (PLVB only) and *env*). Primer sequences and genome coordinates are listed in Table A2.

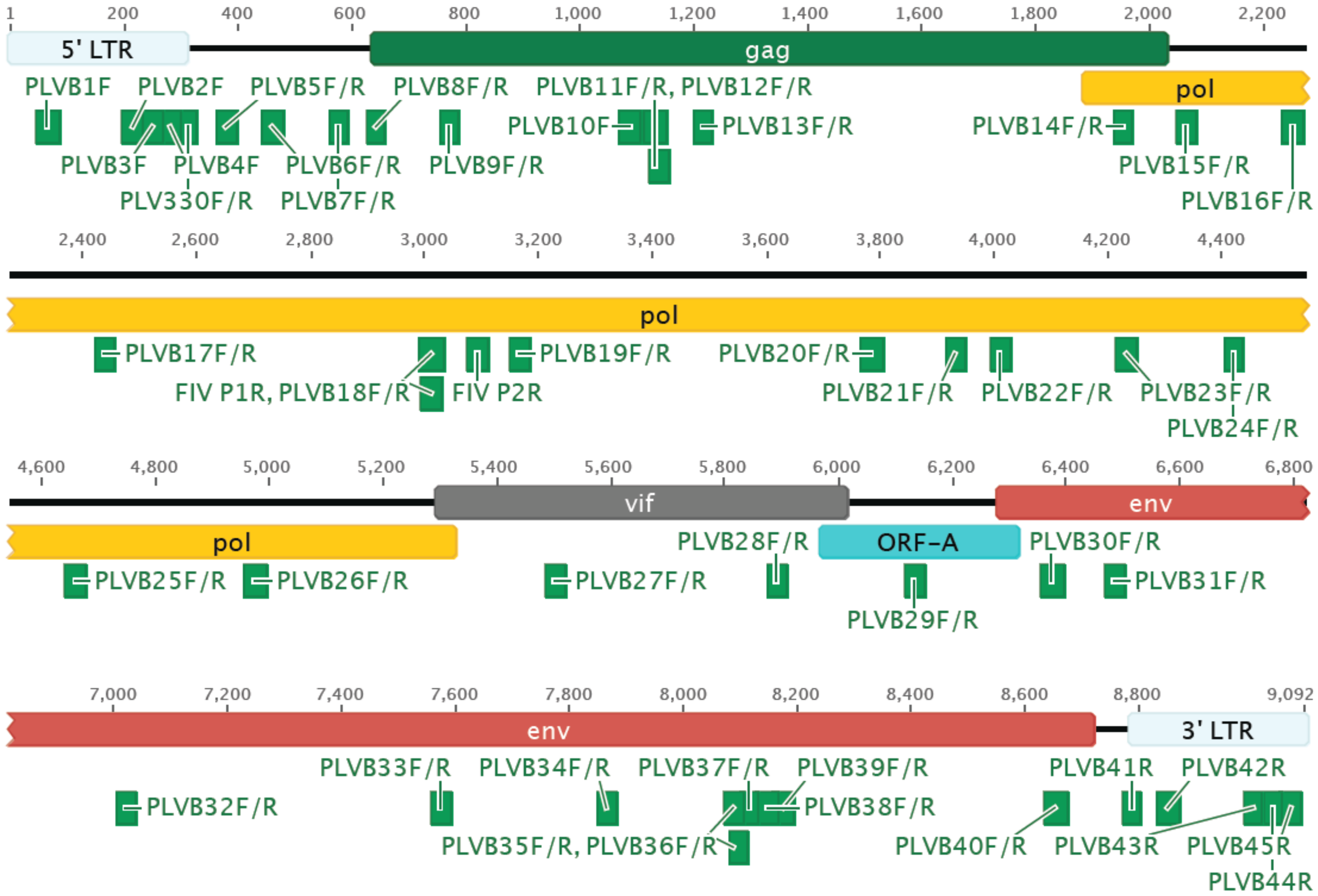
1
200
400
600
800
1,000
1,200
1,400
1,600
1,800
2,000
2,200
5' LTR
gag
pol
PLVB1F
PLVB2F
PLVB5F/R
PLVB8F/R
PLVB11F/R, PLVB12F/R
PLVB10F
PLVB13F/R
PLVB14F/R
PLVB3F
PLVB4F
PLVB6F/R
PLVB9F/R
PLVB15F/R
PLV330F/R
PLVB7F/R
PLVB16F/R
2,400
2,600
2,800
3,000
3,200
3,400
3,600
3,800
4,000
4,200
4,400
pol
PLVB17F/R
PLVB19F/R
PLVB20F/R
FIV P1R, PLVB18F/R
FIV P2R
PLVB21F/R
PLVB22F/R
PLVB23F/R
PLVB24F/R
4,600
4,800
5,000
5,200
5,400
5,600
5,800
6,000
6,200
6,400
6,600
6,800
vif
env
pol
PLVB28F/R
ORF-A
PLVB30F/R
PLVB25F/R
PLVB26F/R
PLVB27F/R
PLVB31F/R
PLVB29F/R
7,000
7,200
7,400
7,600
7,800
8,000
8,200
8,400
8,600
8,800
9,092
env
3' LTR
PLVB33F/R
PLVB34F/R
PLVB37F/R
PLVB39F/R
PLVB41R
PLVB42R
PLVB32F/R
PLVB38F/R
PLVB35F/R, PLVB36F/R
PLVB40F/R
PLVB43R
PLVB45R
PLVB44R

Figure A1b

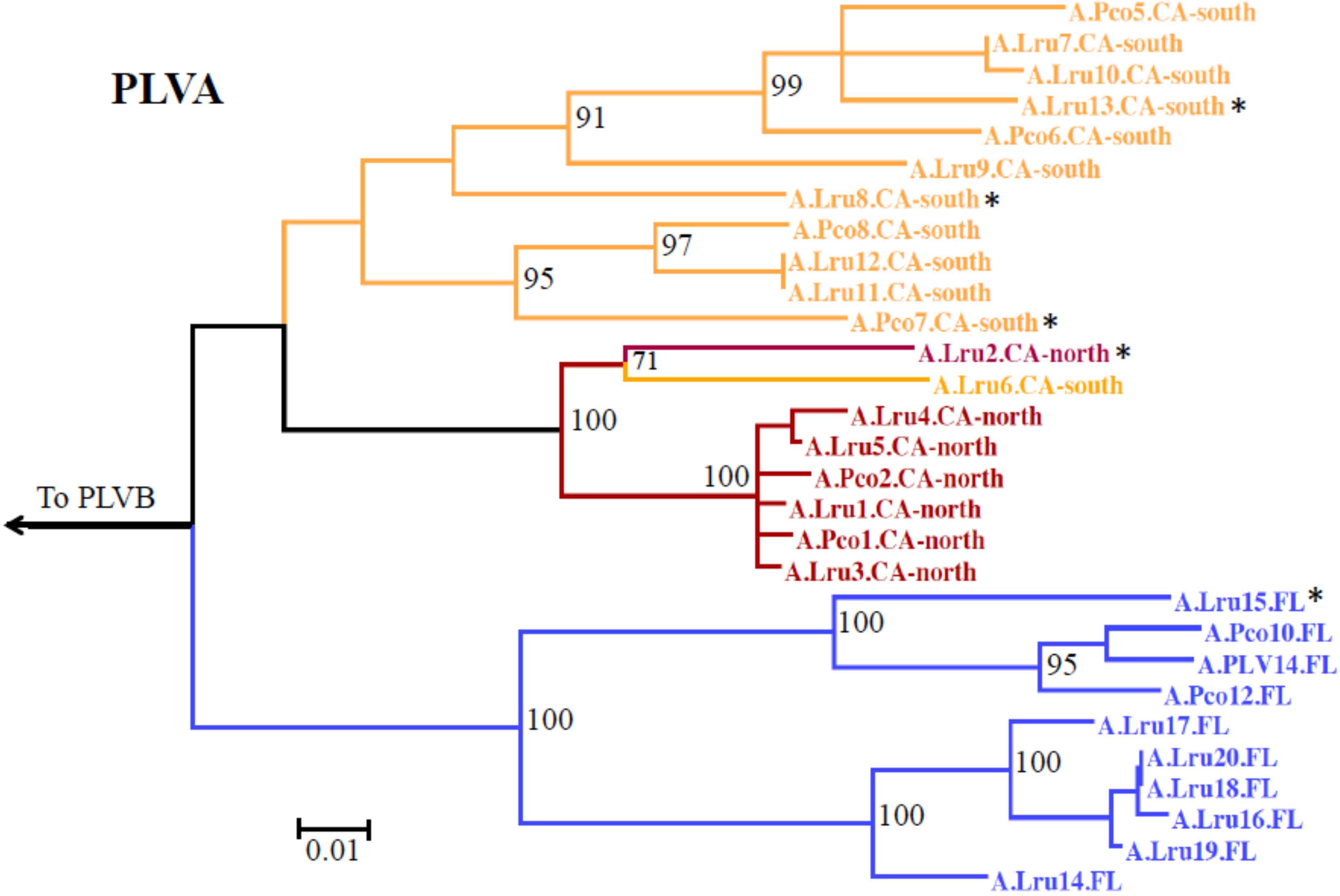

Figure A2a: Phylogenetic trees depicting alternative evolutionary histories of genomic regions resulting from recombination between isolates from different viral lineages. Neighbor-joining trees were constructed from all non-recombinant genome regions (Figure 4). One tree from each clade is presented here to illustrate the effect of recombination on viral genetic diversity. The trees represent the following genome regions: PLVA – *gag* 905-1368 (520bp); PLVB – *env* 6529 – 7122 (620bp). Bootstrap values estimated from 1000 replicates are labeled on nodes with greater than 70% cluster support. Isolates labeled with * are likely recombinant viruses because their position on this tree is incongruent (supported by bootstrap values > 70) with their position in Figure 2.

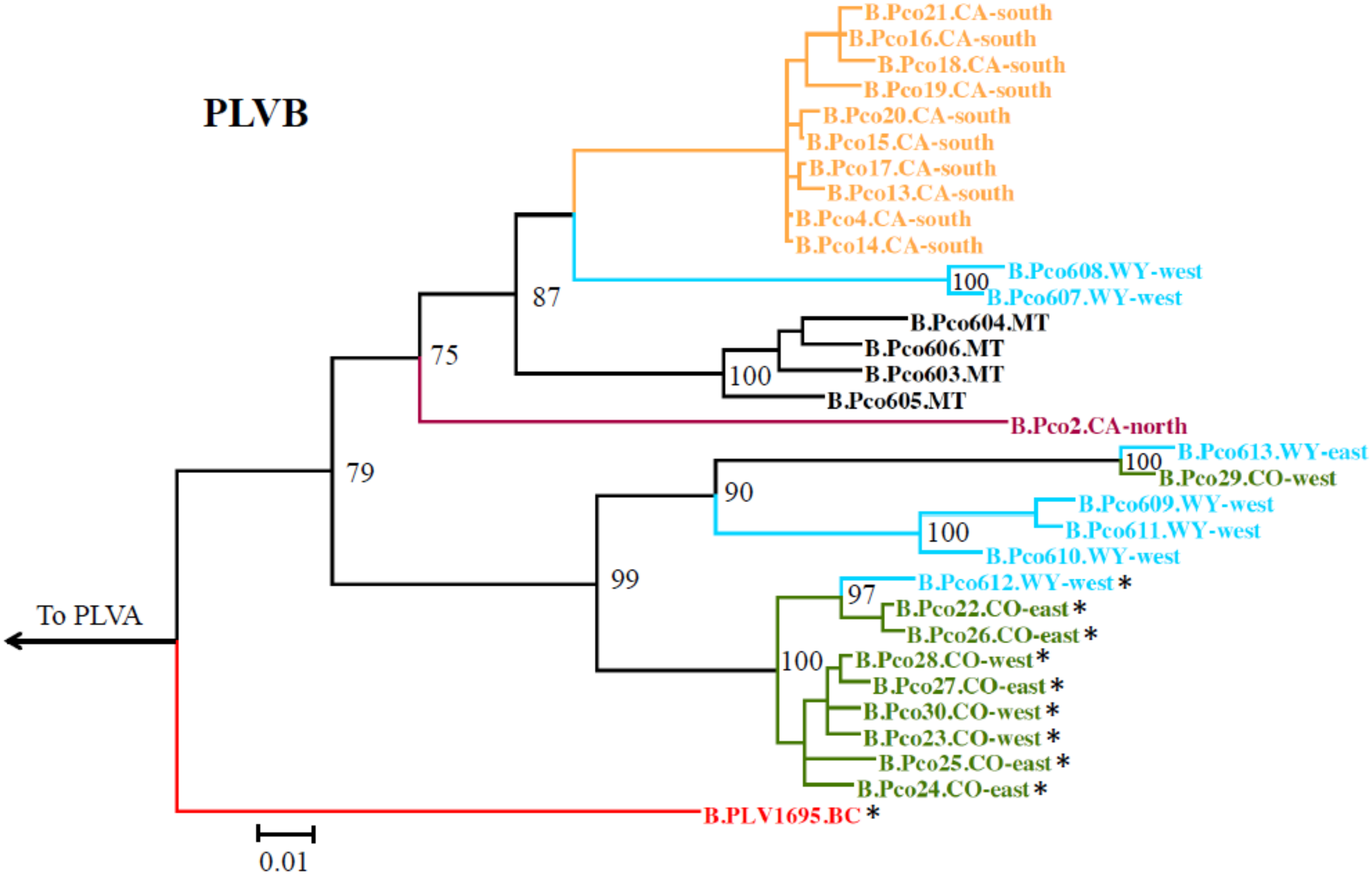
PLVB
B.Pco21.CA-south
B.Pco16.CA-south
B.Pco18.CA-south
B.Pco19.CA-south
B.Pco20.CA-south
B.Pco15.CA-south
B.Pco17.CA-south
B.Pco13.CA-south
B.Pco4.CA-south
B.Pco14.CA-south
B.Pco608.WY-west
100
B.Pco607.WY-west
87
B.Pco604.MT
B.Pco606.MT
75
100
B.Pco603.MT
B.Pco605.MT
B.Pco2.CA-north
B.Pco613.WY-east
100
B.Pco29.CO-west
79
90
B.Pco609.WY-west
B.Pco611.WY-west
100
B.Pco610.WY-west
99
B.Pco612.WY-west *
97
B.Pco22.CO-east *
B.Pco26.CO-east *
To PLVA
100
B.Pco28.CO-west *
B.Pco27.CO-east *
B.Pco30.CO-west *
B.Pco23.CO-west *
B.Pco25.CO-east *
B.Pco24.CO-east *
B.PLV1695.BC *
0.01

Figure A2b

Table A1: Demographic information for all bobcat (Lru) and puma (Pco) samples included in this study. GenBank accession numbers for previously published sequences are shown in parentheses.

PLVA Isolates				
Isolate Identifier	**Location**	**Year Collected**	**Sex**	**Age**
Lru1	CA-north	1996	M	A
Lru2	CA-north	2001	M	A
Lru3	CA-north	2001	M	A
Lru4	CA-north	2002	M	A
Lru5	CA-north	2002	F	Y
Lru6	CA-south	2003	M	A
Lru7	CA-south	2005	M	A
Lru8	CA-south	2006	M	A
Lru9	CA-south	2007	M	A
Lru10	CA-south	2006	M	A
Lru11	CA-south	2009	M	A
Lru12	CA-south	2009	F	A
Lru13	CA-south	2009	M	A
Lru14	FL	1984	-	-
Lru15	FL	2007	F	A
Lru16	FL	2010	M	A
Lru17	FL	2010	M	A
Lru18	FL	2010	M	A
Lru19	FL	2010	M	A
Lru20	FL	2010	M	A
Pco1	CA-north	2004	F	A
Pco2*§	CA-north	2002	M	A
Pco3*	CA-north	2009	M	A
Pco4*§	CA-south	2006	M	A
Pco5	CA-south	2004	F	A
Pco6	CA-south	2003	M	A
Pco7	CA-south	2004	F	A
Pco8	CA-south	2002	F	A
Pco9*	CA-south	2010	M	A
Pco10*	FL	1991	-	-
Pco11*	FL	1987	-	-
Pco12*	FL	1991	-	-
PLV14 (U03982)	FL	1991	-	-

* - only a portion of the PLVA genome was sequenced

§ - co-infected with both PLVA and PLVB viruses

Table A1 continued

PLVB Isolates				
Isolate Identifier	**Location**	**Year Collected**	**Sex**	**Age**
Pco2§	CA-north	2002	M	A
Pco4§	CA-south	2006	M	A
Pco13	CA-south	2005	F	A
Pco14	CA-south	2002	F	A
Pco15	CA-south	2002	M	A
Pco16	CA-south	2011	M	-
Pco17	CA-south	2003	M	A
Pco18	CA-south	2002	F	A
Pco19	CA-south	2003	F	A
Pco20	CA-south	2003	F	A
Pco21	CA-south	2003	F	A
Pco22	CO-east	2009	M	A
Pco23	CO-west	2010	F	A
Pco24	CO-east	2010	F	Y
Pco25	CO-east	2010	F	A
Pco26	CO-east	2010	F	A
Pco27	CO-east	2008	F	A
Pco28	CO-west	2009	M	A
Pco29	CO-west	2009	F	A
Pco30	CO-west	2009	M	A
PLV1695 (DQ192583)	BC	1995	-	-
Pco603 (EF455603)	MT	2001	-	-
Pco604 (EF455604)	MT	2002	-	-
Pco605 (EF455605)	MT	2001	-	-
Pco606 (EF455606)	MT	2001	-	-
Pco607 (EF455607)	WY-west	1992	-	-
Pco608 (EF455608)	WY-west	1992	-	-
Pco609 (EF455609)	WY-west	2004	-	-
Pco610 (EF455610)	WY-west	2003	-	-
Pco611(EF455611)	WY-west	2001	-	-
Pco612 (EF455612)	WY-west	2001	-	-
Pco613 (EF455613)	WY-east	2001	-	-
§ - co-infected with both PLVA and PLVB viruses				

Table A2: PCR primers used to amplify and sequence PLVA and PLVB isolates. Primer sequences are listed in the 5’- to 3’- direction. The location of the 5’- base (forward primers) or 3’-base (reverse primers) is listed relative to the reference sequence for each clade (PLV14 – PLVA; PLV1695 - PLVB). Primers denoted with 1 and 2 were originally published in (4) and (1) respectively. All other primers were designed in this study.

	PLVA	
Primer	Sequence	Location
PLVA1F	AAC TAG CTT AAC CGY AAA CCG CA	100
PLVA2F	CCA CAT CCT ATA GAA ATG ACA AWA AGA	131
PLVA3F	AGA TCC TCA GGT ATG CTT TAA TAA AGA G	208
PLVA4F	GTG AAC CTT GGT GGC TGC CTG	241
PLVA5F	GGC CAG AAA CYC TGC AGT TGG	296
PLVA6F	GCT TGG TTG AAG AGA TAC AAA CTG	482
PLVA6R	CAG TTT GTA TCT CTT CAA CCA AGC	
PLVA7F	TAG GTT CTC AAG CGG GAC ACC A	554
PLVA7R	TGG TGT CCC GCT TGA GAA CCT A	
PLVA8F	GAT GGA AAA RGC TAG AGG AGG	1089
PLVA8R	CCT CCT CTA GCY TTT TCC ATC	
PLVA9F	GAR GAA GCA GTN TTA TGG TTT ACT G	1123
PLVA9R	AGT AAA CCA TAA GAC TGC TTC	
PLVA10F	AAT GCW AAT GGR GAG TGT AGA ARR GC	1573
PLVA10R	GCY YTT CTA CAC TCY CCA TTW GCA TT	
PLVA11F	GGG GAA TAG TAT ATT TGA TGG ATA TCA	2172
PLVA11R	GGG GTG ATA TCC ATC AAA TAT ACT ATT	
PLVA12F	GGC CAT TGT TTT ATT TGA GGN CC	2439
PLVA12R	GGN CCT CAA ATA AAA CAA TGG CC	
PLVA13F	TTC WGT AAR AGC TTC TAT YTT TTC ATT WGT TA	2464
PLVA13R	CAA ATG AAA AAA TAG AAG CTT TAA C	
PLVA14F	GAA CCY CCY TAT AAR TGG ATG GGA TAT	3054
PLVA14R	ATA TCC CAT CCA TTT ATA GGG RGG TTC	
PLVA15F	GGN TRG GAA GRA TGA ATA GRC AAA AGA ARA	3553
PLVA15R	TYT TCT TTT GYC TAT TCA TYC TTC CYA NCC	
PLVA16F	TTA GGA GGW GTA ATT GAT CAA GGA T	4362
PLVA16R	ATC CTT GAT CAA TTA CWC CTC CTA A	
PLVA17F	TAA ART TAG GWG AAG GDA TAT GGC ARA T	4744
PLVA17R	ATY TGC CAT ATH CCT TCW CCT AAY TTT A	

Table A2 Continued

PLVA (continued)

Primer	Sequence	Location
PLVA18F	GAA AGG GTG GAT TAG GGG GTA TYA CAC	5143
PLVA18R	GTG TRA TAC CCC CTA ATC CAC CCT TTC	
PLVA19F	CWT GGG AWT ATT GTG GAG ATT GTT GG	6919
PLVA19R	CCA ACA ATC TCC ACA ATA WTC CCA WG	
PLVA20F	GGA TYG GAC CTG AAG AAG GAG AAA TG	6281
PLVA20R	CAT TTC TCC TTC TTC AGG TCC RAT CC	
PLVA21F	GCT ATA GAT CCT CCT TGG GTK ATT CC	6766
PLVA21R	GGA ATA AYC CAA GGD GGA TCT AWA GC	
PLVA22F	TGT TCA GGR ATA CCG GGA GTA GAT	7450
PLVA22R	ATC TAC TCC CGG TAT YCC TGA ACA	
envfw201[1]	TTT CTC ATG TTC CTT GAA TGG TAC	7761
envfw202[1]	TGG TAC ATT CTG GGT GTT TAA ATC	7779
PLVA23F	GTC ACT GCT GGR ATG ATW GGG	8047
PLVA23R	CCC WAT CAT YCC AGC AGT GAC	
PLVA24F	GCA CTG CAG CCC TGR CGG TAT C	8078
PLVA24R	GAT ACC GYC AGG GCT GCA GTG C	
PLVA25F	GCG ACT CAA WGA GWT AAT GCT CC	8103
PLVA25R	GGA GCA TTA WCT CWT TGA GTC GC	
PLVA26F	GGA TTG TTC AGA GGA GAC TGC	8165
PLVA26R	GCA GTC TCC TCT GAA CAA TCC	
PLVA27F	CAG AGG AGA CTG CAA GAT GCA G	8173
PLVA27R	CTG CAT CTT GCA GTC TCC TCT G	
envrv201[1]	GCA TCA GAG AGT GAC CAA AAT AG	8214
envrv202[1]	CAA TAC CAA TTA AGT GGA ATG TG	8254
PLVA28R	CTT CCC AGT CCA CCC TTT CTT CTT	8773
PLVA29R	TGC GGT TTR CGG TTA AGC TAG TT	8889
PLVA30R	TCT TAT TGT CAT TTC TAT AGG ATG TGG	8920
PLVA31R	CTC TTT ATT AAA GCA TAC CTG AGG ATC T	8997
PLVA32R	CAG GCA GCC ACC AAG GTT CAC	9033

Table A2 continued

PLVB		
Primer	Sequence	Location
PLVB1F	CTA GCT TTR RCC RYA AAC CGC AAR T	51
PLVB2F	CAG RCT GYC CCT CAG GTA GAA TAA A	201
PLVB3F	AAC CCT GAC WTC TGC CTG AGA	240
PLVB4F	TCT TAT GTG GGT CTA AGG RAT CCG	268
PLVB5F	CCC AGT CAA GAG TAA GGC TTG GTA G	367
PLVB5R	CTA CCA AGC CTT ACT CTT GAC TGG G	
PLVB6F	CTT GGT TGA AGA GAC ACT GRC TG	447
PLVB6R	CAG YCA GTG TCT CTT CAA CCA AG	
PLVB7F	CTT CAC GGA TCW TCA AGC CAG	565
PLVB7R	CTG GCT TGA WGA TCC GTG AAG	
PLVB8F	CTG TCT GTC ATG GGG AAT GAG T	630
PLVB8R	ACT CAT TCC CCA TGA CAG ACA G	
PLVB9F	TTG CCT TGG TAG CTA CAG GAC	760
PLVB9R	GTC CTG TAG CTA CCA AGG CAA	
PLVB10F	ACA GCT TTC AAY CCT AGA ACA GTA GC	1074
PLVB11F	AGA RGG AAT ACA TAG TGA AGA RGC CAT	1119
PLVB11R	ATG GCY TCT TCA CTA TGT ATT CCY TCT	
PLVB12F	ATA CAT AGT GAA GAA GCC ATT CTG	1128
PLVB12R	CAG AAT GGC TTC TTC ACT ATG TAT	
PLVB13F	TGT CAG CTC CAG GAT GTG CT	1207
PLVB13R	AGC ACA TCC TGG AGC TGA CA	
PLVB14F	CCA GAG AAA TCA AAT GCA GGT	1943
PLVB14R	ACC TGC ATT TGA TTT CTC TGG	
PLVB15F	CGT GTT GAG GCC TGG ATA AAT G	2054
PLVB15R	CAT TWA TCC AGG CCT CAA CWC G	
PLVB16F	ACA GAA AAY GAA GGA ARA TGT TGT AA	2240
PLVB16R	TTA CAA CAT YTT CCT TCR TTT TCT GT	
PLVB17F	TTC TGC TGA TAA TGG CCA TTG TT	2427
PLVB17R	AAC AAT GGC CAT TAT CAG CAG AA	

Table A2 continued

PLVB (continued)		
Primer	Sequence	Location
PLVB18F	GAT TTG AAA CWC CAG ARG ATA AGC T	3000
PLVB18R	AGC TTA TCY TCT GGW GTT TCA AAT C	
PLVB19F	AAT TGG GCM ACT CAA ATA ATA GG	3155
PLVB19R	CCT ATT ATT TGA GTK GCC CAA TT	
PLVB20F	GCA AAT WAT GGA RAT AGA AGG ATC TAA TC	3772
PLVB20R	GAT TAG ATC CTT CTA TYT CCA TWA TTT GC	
PLVB21F	CCT TAT GGA ARG AGA TTA TAG AAG A	3921
PLVB21R	TCT TCT ATA ATC TCY TTC CAT AAG G	
PLVB22F	CCW GGA AAT AAR GAA ATW GAT GA	4001
PLVB22R	TCA TCW ATT TCY TTA TTT CCW GG	
PLVB23F	AAA GGW TTA GAT GTV YTA GGA GGA GT	4220
PLVB23R	ACT CCT CCT ARB ACA TCT AAW CCT TT	
PLVB24F	ACA AGG GTT TGG RAG YAC AGG	4411
PLVB24R	CCT GTR CTY CCA AAC CCT TGT	
PLVB25F	ATA GAT TGY ACA CAT WTA GAA GGA CA	4646
PLVB25R	TGT CCT TCT AWA TGT GTR CAA TCT AT	
PLVB26F	AGT RGA AAC TTT ACA RGC RGC AGT AG	4961
PLVB26R	CT ACT GCT GCY TGT AAW GTT TCY ACT TC	
PLVB27F	GAA TTA GTA GCA GGR ACA GGR C	5493
PLVB27R	GGY CCT GTY CCT GCT ACT AAT TC	
PLVB28F	TCY TGG TAT TGT AAA CCT YCT TAC AGG	5880
PLVB28R	YCT GTA AGG RGG TTT ACA ATA CCA	
PLVB29F	TTA GAA TTT GAR GAR GCA ATA GA	6122
PLVB29R	TCT ATT GCY TCY TCA AAT TCT AA	
PLVB30F	GAG AAG ATC ARA GRA TCC CWT CAG GAA	6362
PLVB30R	TTC CTG AWG GGA TYC TYT GAT CTT CTC	
PLVB31F	ACA AAT RCT GGA AGG AGG TGA GTT	6474
PLVB31R	AAC TCA CCT CCT TCC AGY ATT TGT	

Table A2 continued

PLVB (continued)

Primer	Sequence	Location
PLVB32F	TGG AGT RTD GCT TGG TGG ACA TG	7012
PLVB32R	CAT GTC CAC CAA GCH AYA CTC CA	
PLVB33F	GAG AAA TAT GCA CTC AGC CRA CT	7562
PLVB33R	AGT YGG CTG AGT GCA TAT TTC TC	
PLVB34F	ATG AAT AAT GCA TCT TGG AAT TGG	7855
PLVB34R	CCA ATT CCA AGA TGC ATT ATT CAT	
PLVB35F	ACC TCG GTG GCA GGG CTG ATA	8080
PLVB35R	TAT CAG CCC TGC CAC CGA GGT	
PLVB36F	TGG CRG GGC TGA TAG GAG CA	8087
PLVB36R	TGC TCC TAT CAG CCC YGC CA	
PLVB37F	ACC ACT GGC ACC ACG GCC TT	8107
PLVB37R	AAG GCC GTG GTG CCA GTG GT	
PLVB38F	CTC AGA ACY TAA GAG GGA TTA TGC T	8135
PLVB38R	AGC ATA ATC CCT CTT ARG TTC TGA G	
PLVB39F	CAA ACA GAR ATA GAT GAG CAA ACC TT	8164
PLVB39R	AAG GTT TGC TCA TCT ATY TCT GTT TG	
PLVB40F	ATG GCM AAT CTG ATA GAA ATA AAA GAA G	8641
PLVB40R	CTT CTT TTA TTT CTA TCA GAT TKG CCA T	
PLVB41R	CAT YCC TCC CAG TCY ACC CTT	8777
PLVB42R	CGC AYT TGC GGT TTR YGG YYA AGC TAG	8837
PLVB43R	TTT ATT CTA CCT GAG GGR CAG	8991
PLVB44R	TCT CAG GCA GAW GTC AGG GTT	9026
PLVB45R	CGG ATY CCT TAG ACC CAC ATA AG	9054

PLVA AND PLVB

Name	Sequence	Location
PLV330F	GCA GTT GGC GCC CGA ACA G	309
PLV330R	CCT GTT CGG GCG CCA ACT G	
FIV P1F[2]	TGG CCW YTA WCW AAT GAA AAR ATW GAA	2457
FIV P2F[2]	TGA AAA RAT WGA AGC HTT AAC AGA MAT AG	2471
FIV P1R[2]	GTA ATT TRT CTT CHG GNG TYT CAA ATC CCC	3019
FIV P2R[2]	GTA TTY TCT GCY TTT TTC TTY TGT CTA	3569

Table A3: Detailed genetic diversity, natural selection, and recombination results for all viral proteins studied.

Viral protein	Protein Length (amino acids)		Pairwise Identity		% Invariant Sites		Recombination Breakpoints[1]		Sites Under + Selection[2]		Sites Under - Selection[2]	
	PLVA	PLVB	PLVA	PLVB	PLVA	PLVB	PLVA	PLVB	PLVA	PLVB	PLVA	PLVB
Gag	**479-485**	**461-462**	**91.5**	**94.9**	**77.0**	**80.8**			**4 (0.8)**	**8 (1.7)**	**257 (53.0)**	**222 (48.1)**
MA	136	134	89.2	94.9	72.1	77.6			2 (1.5)	4 (3.0)	66 (48.5)	52 (38.8)
CA	234	232	96.3	98.9	88.5	90.9	905		0	0	144 (61.5)	132 (56.9)
NC	83-90	78-79	83.3	84.5	58.1	58.8	1368	1262*	2 (2.2)	4 (5.1)	38 (41.3)	31 (39.2)
post-NC	22-25	17	90.4	87.4	72.0	70.6	1820		0	0	9 (39.1)	7 (41.2)
Pol	**1135-1140[3]**	**1142-1146**	**88.2**	**89.7**	**67.2**	**64.4**			**17 (1.4)**	**14 (1.2)**	**593 (50.2)**	**591 (51.6)**
pre-PR	32-34	40-41	76.2	76.0	44.1	31.7			4 (11.8)	5 (12.2)	2 (5.9)	2 (4.9)
PR	119-120	117	91.4	89.3	74.2	65.0	2128		4 (3.3)	1 (0.9)	64 (53.3)	54 (46.2)
RT	442	449	88.6	90.9	67.6	67.7	2665		4 (0.9)	5 (1.1)	230 (52.0)	250 (55.7)
RNaseH	112	112	86.0	91.4	63.4	67.0			2 (1.8)	0	66 (58.9)	59 (52.7)
dUTPase	133	133	87.9	87.8	67.7	63.2	4207		1 (0.8)	1 (0.8)	74 (55.6)	70 (52.6)
IN	297-299	290-294	88.4	90.0	67.6	63.3		4696	2 (0.7)	2 (0.7)	157 (52.5)	156 (53.1)
Vif	**277**	**232-245**	**88.8**	**85.7**	**65.3**	**57.4**		5849	**5 (1.8)**	**5 (2.0)**	**142 (51.4)**	**93 (38.0)**
Env	**834-841**	**809-813**	**83.2**	**87.4**	**55.2**	**57.8**			**16 (1.9)**	**23 (2.8)**	**400 (46.9)**	**325 (39.9)**
leader	172-175	162-165	78.3	84.5	51.4	50.3	6551, 6758	6529	4 (2.3)	5 (3.0)	80 (45.7)	61 (37.0)
SU	410-416	425-429	86.0	88.5	57.4	59.3		7122, 7633, 7779, 7995	8 (1.9)	12 (2.8)	219 (51.8)	191 (44.5)
TM	249-254	219-220	81.8	87.3	53.3	60.5	8337*, 8643*	8575	4 (1.6)	6 (2.7)	101 (39.6)	73 (33.2)
Overall	2724-2739	2645-2664	87.2	89.4	65.1	64.2	10 breakpoints	9 breakpoints	42 (1.5)	50 (1.9)	1392 (50.8)	1231 (46.2)

1 - Numbers represent the nucleotide position in either the PLVA (PLV14) or PLVB (PLV1695) reference sequences. All breakpoints significant at alpha = 0.01 except those labled with (*) which are significant at alpha = 0.05.

2 - The number (and %) of sites under selection within each specified genome region.

3 - Numbers do not include a unique 41aa insert in PLV14 RT, which may be an artifact of prolonged cell culture (Langley *et al.*, 1994).

Abbreviations: matrix (MA); capsid (CA), nucleocapsid (NC); protease (PR); reverse transcriptase (RT); deoxy-uridine transferase (dUTPase); integrase (IN); surface (SU); transmembrane (TM)