

Supplementary Material and Methods

Plasmid construction. The reporter pFRT-IRES-HIV was derived from pDual-HIV(-1) (1) that contains the Rluc and Fluc coding sequences separated by HIV-1 frameshift region. Replacement of the frameshift region by the 5'UTR of HIV-1 was made in several steps. First, the AflIII restriction site located near the T7 sequence of pDual-HIV(-1) was deleted to facilitate subsequent cloning. A PCR fragment was amplified using the Δ AflIII-SpeI(+) and Δ AflIII-HindIII(-) primers, which were inserted in the SpeI and HindIII sites of pDual-HIV(-1) to produce pDual-HIV(-1) Δ AflIII. Next, the beginning of the HIV-1 5'UTR contained in pTAR (2) was amplified by PCR using the TAR-Kpn-uag(+) and TAR-Kpn(-) primers and cloned in the KpnI restriction site of pDual-HIV(-1) Δ AflIII located between Rluc and Fluc coding sequences. This PCR introduced three stop codons to terminate the Rluc coding sequence. The rest of the 5'UTR was amplified directly from a proviral molecular clone of HIV-1 group M subtype B (pLAI) (4). The initiation codon for Fluc expression is located within the 5'UTR IRES and the context of the AUG encompassing 30 nt from the Gag sequence was included. A peptide linker (GGGGSGGGGS) was inserted by PCR immediately upstream of the Fluc coding sequence. The first half of the linker was inserted using PCR amplification on pLAI with the 5'UTR-AflIII(+) and 5'UTR-BamHI(-) primers and the second half of the linker with the 5'UTR(+) and Linker-BamHI(-) primers. The linker was cloned in the AflIII and BamHI restriction sites of pDual-HIV(-1) Δ AflIII-TAR to generate pDual-IRES-HIV. This last step removed the frameshift region originally present in pDual-HIV(-1). Finally, we cut the fragment containing the Rluc coding sequence, the 5'UTR region of HIV-1 RNA and the Fluc coding sequence, using PmeI and ApaI restriction sites. This fragment was inserted into pcDNA5FRT (Invitrogen) previously linearized with SciI and ApaI to produce pFRT-IRES-HIV. Prior to this last cloning step, the KpnI and BamHI restriction sites from pcDNA5FRT were eliminated to facilitate subsequent cloning of mutant IRESes. To this end, an oligonucleotide cassette formed by the K7 Δ KpnI Δ BamHI(+) and K7 Δ KpnI Δ BamHI(-) primers was inserted in the HindIII and EcoRV restriction sites of pcDNA5FRT. The different mutants of HIV-1 IRES were made by PCR amplification with four primers. The external primers were Ext-KpnI(+) et Ext-BamHI(-). The details for all the primers used can be found in Supplementary Table 1. The resulting PCR products were cloned in the KpnI and BamHI restriction sites of pFRT-dual-IRES-HIV.

**Supplementary Table I:
Names and Sequences of the Oligonucleotide Primers Used in this Study**

Primer Name	Primer Sequence
ΔAflIII-SpeI (+)	5' GACCCGGGGTACCAAGCTTGAGTTTAAACGCTAGCCAGC' 3
ΔAflIII-HindIII (-)	5' GCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGG' 3
TAR-Kpn-uag (+)	5' GCGTTTAAACTTAAGCTTGGTACCCTAGGGTCTCTCTGGTTAGACCAG' 3
TAR-Kpn (-)	5' CGAGCTCGGTACCAAGCTTTATTGAGGC' 3
5' UTR-AflIII (+)	5' GGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCC' 3
5' UTR-BamHI (-)	5' AAAAAAGGATCCGCTTCCGCCTCCGCCGGTGGCTCTAGCGCTCCCCCGCTTAATACT GACGCTCTCGCACCCATCTCTCTCCC' 3
Linker-BamHI (-)	5' TTTTTTGGATCCTGCTTCCGCCTCCGCCGCTTCCGCCTCCGCCGGTGGCTCTAGCGCT CCC' 3
K7ΔKpnIΔBamHI (+)	5' AGCTTGGTAGCGAGCTCGCATCCACTAGTCCAGTGTGGTGAATTCTGCAGAT' 3
K7ΔKpnIΔBamHI (-)	5' ATCTGCAGAATTCACCACACTGGACTAGTGGATGCGAGCTCGCTACCA' 3
Ext-KpnI (+)	5' GAGCTTCGTGGAGAGAGTGCTG' 3
Ext-BamHI (-)	5' CGTCTTCTTGGATCCTGGTGTCTCCGCCTC' 3
M1-Δloop202-217 (+)	5' GCCCGAACAGGGACTTCCAGAGGAGCTCTCTCGACGC' 3
M1-Δloop202-217 (-)	5' GAGAGAGCTCCTCTGGAAGTCCCTGTTCCGGGCGCC' 3
M2-ΔSL134-178 (+)	5' GCCCGTCTGTTGTGTGACTCTGGTAACAGTGGCGCCCGAACAGGG' 3
M2-ΔSL134-178 (-)	5' CCCTGTTCCGGGCGCCACTGTTACCAGAGTCACACAACAGACGGGC' 3
M3-stem134 (+)	5' CCCTTTTAGTCAGTGTGGAAACCGGGATCCAGTGGCGCCCGAACAGGG' 3
M3-stem134 (-)	5' CCCTGTTCCGGGCGCCACTGGATCCCGGTTTCCACACTGACTAAAAGGG' 3
M4-stem143 (+)	5' GATCCCTCAGACCCTTTTAAGGAGACTTCAAATCTCTAGCAGTGGCGCCCG' 3
M4-stem143 (-)	5' CCACTGCTAGAGATTTTCCACACTCCTTAAAAGGGTCTGAGGGATCTCTAGTTACC' 3
M5-stem143bot (+)	5' GATCCCTCAGACCCTTTTAGTCAGACTTCAAATCTCTAGCAGTGGCGCCCG' 3
M5-stem143bot (-)	5' CCACTGCTAGAGATTTTGAAGTCTGACTAAAAGGGTCTGAGGGATCTCTAGTTACC' 3
M6-stem143up (+)	5' GATCCCTCAGACCCTTTTAAGGAGTGTGGAAAATCTCTAGCAGTGGCGCCCG' 3
M6-stem143up (-)	5' CCACTGCTAGAGATTTTCCACACTCCTTAAAAGGGTCTGAGGGATCTCTAGTTACC' 3
M7-Δloop151 (+)	5' CTAGAGATCCCTCAGAAGTCAGTGTGGAAAATCTCTCTAGC' 3
M7-Δloop151 (-)	5' GATTTTCCACACTGACTTCTGAGGGATCTCTAGTTACC' 3
M8-loop151 (+)	5' CTAGAGATCCCTCAGAGGGAAAAAGTCAGTGTGGAAAATCTCTAGC' 3
M8-loop151 (-)	5' GATTTTCCACACTGACTTTTTCCCTCTGAGGGATCTCTAGTTACCAG' 3
M9-AAA-CCC (+)	5' CCCTTTTAGTCAGTGTGGCCCATCTCTAGCAGTGGCGCC' 3
M9-AAA-CCC (-)	5' GGCGCCACTGCTAGAGATGGGCCACACTGACTAAAAGGG' 3
M10-AAA-GGG (+)	5' CCCTTTTAGTCAGTGTGGGGATCTCTAGCAGTGGCGCC' 3

M10-AAA-GGG (-)	5' GCGCCACTGCTAGAGATCCCCCAGACTGACTAAAAGGG' 3
M11-AAA-UUU (+)	5' CCCTTTTAGTCAGTGTGGTTTATCTCTAGCAGTGGCGCC' 3
M11-AAA-UUU (-)	5' GCGCCACTGCTAGAGATAAACACACTGACTAAAAGGG' 3
M12-BMH (+)	5' GAGCTCTCTCGACGCACCCCTCGGCTTGCTGAAGCGC' 3
M12-BMH (-)	5' GCTTCAGCAAGCCGAGGGGTGCGTCGAGAGAGCTCCTC' 3
M13-BMH (+)	5' GCACGGCAAGAGGCGAGTCCAGGCGACTGGTGAGTACG' 3
M13-BMH (-)	5' CGTACTCACCAGTCGCCTGGACTCGCCTCTTGCCGTGC' 3
M14-BMH (+)	5' GGGAGGCGACTGGTGAGTCGCCTAAAATTTTACTAGCGGAGGCTAG' 3
M14-BMH (-)	5' CTAGCCTCCGCTAGTCAAAAATTTTAGGCGACTCACCAGTCGCCTCCC' 3
M15-LDI (+)	5' CGAGGGGAGGCGACTGGTGCGCACCCAAAAATTTTACTAGC' 3
M15-LDI (-)	5' GCTAGTCAAAAATTTTGGGTGCGCACCCAGTCGCCTCCCCTCG' 3
M16-LDI (+)	5' CGACTGGTGAGTACGCCATCCTTTTTTACTAGCGGAGGC' 3
M16-LDI (-)	5' GCCTCCGCTAGTCAAAAAGGATGGCGTACTCACCAGTCG' 3
M17-LDI (+)	5' GGTGAGTACGCCAAAAATTTTTTCTAGCGGAGGCTAGAAGGAGAG' 3
M17-LDI (-)	5' CTCTCCTTCTAGCCTCCGCTAGAAAAATTTTTTGGCGTACTCACC' 3

Supplementary Results

The 134-178 stem-loop, the 202-217 region and the four-nt bulge in the DIS hairpin, but not the A stretch in loop I, are highly conserved among natural variants of HIV-1 group M subtype B.

The 5'UTR region of HIV-1 is the most conserved region in the HIV-1 genome (3). The HIV-1 5'UTR region used in this study originates from pLAI. We examined the conservation of the different regions which we found to influence the 5'UTR IRES activity among group M subtype B natural variants. These regions of pLAI were aligned with the corresponding regions of HIV-1 group M subtype B variants available in the HIV sequence database of the Los Alamos National Laboratory available at the following URL : <http://www.hiv.lanl.gov/>. When the 134-178 stem-loop (IRENE) from pLAI was aligned with the corresponding region of 97 available variants, we found that it is highly conserved (Supp. Fig. 1A). Most of the differences are located in the upper 7-pyrimidine loop, which did not influence the 5'UTR IRES activity. Twenty-one variants have mutations in the upper stem, but only five of these mutations disrupt a base-pair. The 3A bulge is conserved in all these variants except three. When nt 200-219 of pLAI were aligned with the corresponding region of 129 available variants (Supp. Fig. 1B), this unstructured region was also found to be highly conserved except for the four nt at the 3' end. When nt 236-246 of pLAI were aligned with the corresponding region of 142 available variants, we observed that the four-nt bulge at the beginning of the DIS hairpin (Supp. Fig. 1C) is highly conserved since only four variants differ from pLAI. Finally, nt 301-305 of pLAI, a stretch of five A in the beginning of loop I, were aligned with the corresponding region of 149 available variants (Supp. Fig. 1D). The five A are conserved in 60 variants. However, 62 variants have deletions and/or mutations in this region. Therefore, while the 301-305 segment shows some variability, IRENE, the 202-217 region and the 239-242 bulge are highly conserved.

Supplementary Figure Legends

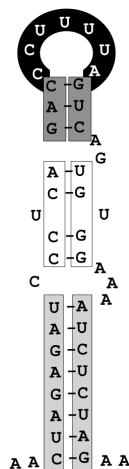
Supplementary FIGURE 1

(A) Sequence alignment of the 134-178 stem-loop. These sequences originated from pLAI and the corresponding regions of HIV-1 group M subtype B variants retrieved from HIV-1 database from Los Alamos National Laboratory. Lines indicate identical bases, R represents A or G; Y represents C or U; B represents C, G or U; N represents any nt. When the region of interest of a variant is identical to pLAI, its name is in bold. (B) Sequence alignment of the unstructured 202-217 segment. (C) Sequence alignment of the four-nt bulge in the DIS hairpin (nt 239-242). (D) Sequence alignment of the beginning of loop I (nt 302-305). In (B), (C) and (D), the region of interest is in gray.

Supplementary References

1. Dulude, D., Berchiche, Y.A., Gendron, K., Brakier-Gingras, L. and Heveker, N. (2006) Decreasing the frameshift efficiency translates into an equivalent reduction of the replication of the human immunodeficiency virus type 1. *Virology*, **345**, 127-136.
2. Gendron, K., Charbonneau, J., Dulude, D., Heveker, N., Ferbeyre, G. and Brakier-Gingras, L. (2008) The presence of the TAR RNA structure alters the programmed -1 ribosomal frameshift efficiency of the human immunodeficiency virus type 1 (HIV-1) by modifying the rate of translation initiation. *Nucleic Acids Res*, **36**, 30-40.
3. Berkhout, B. (1996) Structure and function of the human immunodeficiency virus leader RNA. *Prog Nucleic Acid Res Mol Biol*, **54**, 1-34.
4. Peden, K., Emerman, M. and Montagnier, L. (1991) Changes in growth properties on passage in tissue culture of viruses derived from infectious molecular clones of HIV-1LAI, HIV-1MAL, and HIV-1ELI. *Virology*, **185**, 661-672.

A) The 134-178 stem-loop (IRENE)



pLAI	C	U	CA	GAC	CCUUUUU	GUC	AG	UG	U	GG	AAA	A. UCUCUAG
B. FR. 83.HXB2_LAI_IIIB_BRU.K03455												
B. AU. 86.MBC200.AF042100												
B. AU. 87.MBC925.AF042101												
B. AU.x.1181.AF538302						U	C			A		
B. AU.x.C24.AF538304											G	
B. AU.x.C42.AF538305					AA							
B. AU.x.C76.AF538306												
B. AU.x.C92.AF538307												G
B. BR. 02.02BR011.DQ358809					A	A						
B. BR. 02.02BR013.DQ358810	AC				A							A
B. BR. 03.BREPM1023.EF637057												
B. BR. 03.BREPM1038.EF637048					A	C						
B. BR. 04.BREPM1066.FJ195090					AAC							
B. BR. 04.BREPM1070.FJ195086		U	U		U	A	G			U	G	U
B. BR. 04.BREPM1070.FJ195086					U	A	G			U	G	U
B. BR. 05.BREPM1081.FJ195091					A		C					
B. BR. 05.BREPM1093.FJ195089					A							
B. CA. 96.WC10C_4.AY314056					U							
B. CN. 02.02HNSc11.DQ007903					A	A						
B. CN. 05.05CNHB_hp3.DQ990880					A			U	U			
B. CU. 99.Cu19.AY586542					U	C						
B. CU. 99.Cu43.AY586543					U	C						
B. DE. 86.D31.U43096												
B. DE. 86.HAN.U43141					U							
B. ES. 05.ES.EU786672												
B. ES. 06.ES.EU786676												
B. ES. 06.ES.EU786677												
B. ES. 07.ES.EU786678					A							
B. ES. 07.ES.EU786679					A	AGC						U
B. ES. 07.ES.EU786680					AA		C					
B. ES. 89.U61.DQ854716	UAGAGAUC		U		AA					A		
B. GA. 88.OYI.M26727												
B. GB. 83.CAM1.D10112												
B. GB. 86.GB8.AJ271445										A		
B. GB.x.MANC.U23487												
B. IU. 05.SG1.DQ672623												
B. JP. 00.DR2508.AB289588					CUGG					A		
B. JP. 01.DR388.AB289590					A	A						
B. JP. 04.04JPDR6075B.AB221125												
B. JP. 04.DR6089.AB286955					A	A						
B. JP. 05.DR6538.AB287363							C					
B. JP. 05.DR6737.AB287364					A			C				
B. JP. 05.DR7060.AB287366					AU	G						
B. JP. 05.DR7065.AB287368					U	AUC		C				A
B. JP. 99.DR1348.AB287370												
B. KR. 04.04KMH5.DQ295193					AA							
B. KR. 04.04LHS6.AY839827					A							
B. KR. 04.04LSK7.DQ295192												
B. KR. 04.04WK7_HIV_1_wk.DQ295194												
B. KR. 05.05CSR3.DQ837381					AA							
B. NL. 00.671_00U36.AY423387												
B. NL. 86_3202A21.U34604										A		
B. NL. 96.H434_42_A1.AY970948										A		
B. RU. 04.04RU128005.AY682547											R	
B. RU. 04.04RU129005.AY751406					A							
B. UW. 94.UWCYS.AF086817					A							
B. US. 00.14294_1.DQ853436					U				U	C		A
B. US. 00.ES1_20.EF363123												
B. US. 04.ES10_53.EF363127												
B. US. 04.ES4_24.EF363124												
B. US. 04.ES8_43.EF363126					A							
B. US. 83.5018_83.AY835777												
B. US. 83.5157_83.AY835781												
B. US. 83.RF.M17451					UC							
B. US. 83.SF2.K02007												A
B. US. 84.5019_84.AY835779												
B. US. 84.MNCG.M17449					U					G		
B. US. 84.NY5CG.M38431												
B. US. 84.SF33.AY352275												
B. US. 85.5077_85.AY835769												
B. US. 85.Ba_L.AB221005												
B. US. 86.5084_86.AY835775												
B. US. 86.5096_86.AY835749												
B. US. 86.5127_86.AY835774												
B. US. 86.AD87.AF004394												
B. US. 86.YU_2.M93258												
B. US. 87.5113_87.AY835758												
B. US. 87.BC.L02317					U							
B. US. 88.5160_88.AY835763												
B. US. 88.WR27.AF286365												
B. US. 89.P896.U39362					A	CC			U			A
B. US. 90.WCIPR.U69591												
B. US. 90.WEAU160.U21135					A							
B. US. 91.5048_91.AY835761												
B. US. 91.DH12_3.AF069140					A							
B. US. 93.WCD32P0793.DQ487188					AAA	A						
B. US. 93.WCM32P0793.DQ487190					AA							
B. US. 94.5082_94.AY835773												
B. US. 95.5073_95.AY835768												
B. US. 96.5155_96.AY835753												
B. US. 97.ARES2.AB078005					A							
B. US. 98.15384_1.DQ853463									U	C		A
B. US. 98.98USHVTN1925c1.AY560107												
B. US. 98.98USHVTN3605c9.AY560108												
B. US. 98.98USHVTN8229c6.AY560109					A					A		A
B. US. 98.98USHVTN941c1.AY560110					A					A		A
B. US. 98.WC3_0498_4.EF175212					U							A
B. ZA. 03.03ZAPS045MB2.DQ396398					U	AA				A	AA	A

B) The 202-217 segment

	pLAI	UU	GAAAG	CGAAAG	GGAA	AC
B.FR.83.HXB2_LAI_IIIB_BRU.K03455	C-	----	----	----	----	----
B.AU.86.MBC200.AF042100	--	----	----	-A--	----	----
B.AU.87.MBC925.AF042101	--	-U--	----	A----	----	----
B.AU.95.MBCC54.AF042103	--	----	----	A----	----	----
B.AU.96.MBCD36.AF042105	--	----	----	----	----	----
B.AU.x.1181.AF538302	--	----	----	A----	----	----
B.AU.x.C24.AF538304	--	----	----	A----	----	----
B.AU.x.C42.AF538305	CG	----	----	AA--	----	----
B.AU.x.C76.AF538306	--	----	----	A----	----	----
B.AU.x.C92.AF538307	--	----	----	A----	----	----
B.BR.02.02BR008.DQ358808	CU	----	U----	UAG--	----	----
B.BR.02.02BR011.DQ358809	--	----	----	UAG--	----	----
B.BR.02.02BR013.DQ358810	UA	----	----	UAG--	C-	----
B.BR.03.BREPM1023.EF637057	C-	----	----	-AG--	----	----
B.BR.03.BREPM1024.EF637056	--	----	----	UAG--	----	----
B.BR.03.BREPM1027.EF637054	--	----	----	UA--	----	----
B.BR.03.BREPM1033.EF637050	-C	----	----	UAG--	----	----
B.BR.03.BREPM1038.EF637048	--	----	----	UA-C	G-	----
B.BR.03.BREPM1040.EF637047	-A	----	A----	UAG--	----	----
B.BR.03.BREPM2012.EF637046	--	----	----	UA--	G-	----
B.BR.04.BREPM1066.FJ195090	-C	----	-A----	UAG--	----	----
B.BR.04.BREPM1070.FJ195086	--	----	-A----	G----	U AAG--	----
B.BR.05.BREPM1081.FJ195091	--	----	-A----	UA--	----	----
B.BR.05.BREPM1084.FJ195088	--	----	-G----	UAG--	----	----
B.BR.05.BREPM1093.FJ195089	-C	----	----	UAG--	----	----
B.CA.96.WC10C.4.AY314056	--	----	----	A----	----	----
B.CN.01.CNHN24.AY180905	-A	----	----	U--G	----	----
B.CN.02.02HNsc11.DQ007903	C-	----	----	U--G	----	----
B.CN.05.05CNHB_hp3.DQ990880	CG	----	----	U--G	----	----
B.CN.x.RL42.U71182	--	----	----	U--G	----	----
B.CU.99.Cu43.AY586543	--	----	U----	UAG--	----	----
B.DE.86.D31.U43096	--	----	U----	UA-G	----	----
B.DE.86.HAN.U43141	--	----	----	UAG--	----	----
B.DK.01.CTL_016.EF514704	CG	----	----	A----	----	----
B.DK.01.CTL_017.EF514705	--	----	----	A----	----	----
B.DK.01.CTL_018.EF514706	--	----	----	A----	----	----
B.DK.01.CTL_030.EF514708	GC	----	----	UAG--	----	----
B.DK.01.CTL_033.EF514709	YU	A----	----	A----	----	----
B.DK.04.PMVL_012.EF514699	--	----	----	A----	----	----
B.DK.04.PMVL_013.EF514700	--	----	----	A----	----	----
B.DK.04.PMVL_018.EF514697	--	----	----	A----	----	----
B.DK.04.PMVL_025.EF514702	--	----	----	A----	----	----
B.DK.04.PMVL_027.EF514698	CU	----	----	A----	----	----
B.DK.04.PMVL_039.EF514703	--	----	U----	A----	----	----
B.DK.04.PMVL_049.EF514701	--	----	----	UAG--	----	----
B.ES.05.ES.EU786672	--	----	-A----	A----	----	----
B.ES.06.ES.EU786675	CC	----	----	UAG--	----	----
B.ES.06.ES.EU786676	--	----	----	A----	----	----
B.ES.06.ES.EU786677	CC	----	U----	UUG--	----	----
B.ES.07.ES.EU786678	CG	----	----	A----	----	----
B.ES.07.ES.EU786679	--	----	----	----	----	----
B.ES.07.ES.EU786680	-C	----	U----	A--G	----	----
B.ES.89.U61.DQ854716	--	----	----	A----	----	----
B.GA.88.OYI.M26727	-C	U----	U----	U--G	----	----
B.GB.83.CAM1.D10112	----	-A----	----	UAG--	----	----
B.GB.86.GB8.AJ271445	--	----	U----	UUG--	----	----
B.GB.x.MANC.U23487	-C	----	----	UAG--	----	----
B.HK.x.HK003.FJ460500	C-	----	----	A--Δ	----	----
B.HK.x.HK004.FJ460501	-C	----	----	A----	----	----
B.IT.05.SGI.DQ672623	-C	----	----	A----	----	----
B.JP.00.DR2508.AB289588	-C	----	----	UAG--	----	----
B.JP.01.DR388.AB289590	--	----	----	UAG--	----	----
B.JP.04.04JPDR6075B.AB221125	GC	----	----	UAG--	----	----
B.JP.04.DR6089.AB286955	--	----	----	A----	----	----

B.JP.05.DR6538.AB287363	--	----	U----	UAG--	----	----
B.JP.05.DR6737.AB287364	-C-	----	-A----	C--G	----	----
B.JP.05.DR7060.AB287366	--	----	A----	UAG--	----	----
B.JP.05.DR7065.AB287368	-C-	UG--A	G	U--G	----	----
B.JP.99.DR1348.AB287370	--	----	----	UA--G	----	----
B.KR.04.04KMH5.DQ295193	--	----	----	UAG--	----	----
B.KR.04.04LHS6.AY839827	--	----	----	U--GU	----	----
B.KR.04.04LSK7.DQ295192	--	----	----	UAG--	----	----
B.KR.04.04WK7_HIV_1_wk.DQ295194	--	----	-A----	UAG--	----	----
B.KR.05.05CSR3.DQ837381	--	----	----	-A----	----	----
B.MM.99.msSTD101.AB097870	GC	----	----	UA--G	----	----
B.NL.00.671_00T36.AY423387	--	----	-G--	ΔΔΔ	----	----
B.NL.86.3202A21.U34604	GC	----	----	A----	----	----
B.NL.96.H434_42_A1.AY970948	GC	----	----	UAG--	----	----
B.RU.04.04RU128005.AY682547	YY	----	----	A----	----	----
B.RU.04.04RU129005.AY751406	--	----	U----	----	----	----
B.RU.04.04RU139089.AY751407	--	----	----	UAG--	----	----
B.RU.04.04RU139095.AY819715	--	----	----	UAG--	----	----
B.TW.94.TWCYS.AF086817	-C-	----	-A----	A----	----	----
B.US.00.14294_1.DQ853436	GC	----	----	UAG--	----	----
B.US.00.ES1_20.EF363123	--	----	----	A----	----	----
B.US.04.ES10_53.EF363127	AC	----	----	A----	----	----
B.US.04.ES4_24.EF363124	--	----	----	----	----	----
B.US.04.ES8_43.EF363126	CG	----	----	A----	----	----
B.US.83.5018_83.AY835777	--	----	----	UA--	G-	----
B.US.83.5157_83.AY835781	--	----	----	UA--	G-	----
B.US.83.RF.M17451	CA	----	----	UAG--	----	----
B.US.83.SF2.K02007	GC	----	----	UAG--	----	----
B.US.84.5019_84.AY835779	--	----	----	UA--	G-	----
B.US.84.MNCG.M17449	--	----	----	AA----	----	----
B.US.84.NY5CG.M38431	--	----	-G--	UA--	G-	----
B.US.84.SF33.AY352275	--	----	U----	UUΔ	----	----
B.US.85.5077_85.AY835769	--	----	----	UA--	G-	----
B.US.85.Ba_L.AB221005	--	----	----	A----	----	----
B.US.86.5084_86.AY835775	--	----	----	UA--	G-	----
B.US.86.5096_86.AY835749	--	----	----	----	----	----
B.US.86.5127_86.AY835774	--	----	----	UA--	G-	----
B.US.86.AD87.AF004394	--	----	U----	UAG--	----	----
B.US.86.YU_2.M93258	--	----	----	-A----	----	----
B.US.87.5113_87.AY835758	--	----	----	UA--	G-	----
B.US.87.BC.L02317	CG	----	----	A----	----	----
B.US.88.5160_88.AY835763	--	----	----	UA--	G-	----
B.US.88.WR27.AF286365	-C-	A----	----	UAG--	----	----
B.US.89.F896.U39362	CG	----	----	A----	----	----
B.US.90.US1.AY173952	-C-	----	----	A----	----	----
B.US.90.US2.AY173953	--	----	----	----	----	----
B.US.90.US3.AY173954	-A	----	----	UA--	----	----
B.US.90.US4.AY173955	CC	----	----	UAG--	----	----
B.US.90.WCIPR.U69591	--	----	----	----	----	----
B.US.90.WEAU160.U21135	CC	----	----	----	----	----
B.US.91.5048_91.AY835761	--	----	----	UA--	G-	----
B.US.91.DH12_3.AF069140	CG	----	----	A----	----	----
B.US.93.WCD32P0793.DQ487188	-C-	----	U----	A----	----	----
B.US.93.WCM32P0793.DQ487190	--	----	----	-A----	----	----
B.US.94.5082_94.AY835773	--	----	----	UA--	G-	----
B.US.95.5073_95.AY835768	--	----	----	UA--	G-	----
B.US.96.5155_96.AY835753	--	----	----	UA--	G-	----
B.US.97.ARES2.AB078005	--	----	----	A----	----	----
B.US.98.15384_1.DQ853463	GC	----	----	UA--	----	----
B.US.98.98USHVTN1925c1.AY560107	-C-	----	----	A----	----	----
B.US.98.98USHVTN3605c9.AY560108	--	----	----	A----	----	----
B.US.98.98USHVTN8229c6.AY560109	AA	-UG-G	A-U-G	U--Δ	----	----
B.US.98.98USHVTN941c1.AY560110	CA	----	----	UAG--	----	----
B.US.98.WC3_0498_4.EF175212	-C	----	----	UAG--	----	----
B.ZA.03.03ZAPS045MB2.DQ396398	-C-	----	----	A----	----	----

C) The 4-nt 239-242 bulge

	pLAI	C	GC	AGGA	..	CUCG
B.FR.83.HXB2_LAI_IIIIB_BRU.K03455	-	-	----	..	----	..
B.AU.86.MBC200.AF042100	-	-	----	..	----	..
B.AU.87.MBC925.AF042101	-	-	----	..	----	..
B.AU.95.MBCC54.AF042103	-	-	----	..	----	..
B.AU.96.MBCC98.AF042104	-	-	----	..	----	..
B.AU.96.MBCD36.AF042105	-	-	----	..	----	..
B.AU.x.1181.AF538302	-	-	----	..	----	..
B.AU.x.2870718.AY857022	-	-	----	..	----	..
B.AU.x.C24.AF538304	-	-	----	..	----	..
B.AU.x.C42.AF538305	-	-	-----A----	..	----	..
B.AU.x.C76.AF538306	-	-	----	..	-----U	..
B.AU.x.C92.AF538307	-	-	----	..	----	..
B.AU.x.VH.AF146728	-	-	----	..	----	..
B.BR.02.02BR008.DQ358808	-	-	----	..	----	..
B.BR.02.02BR011.DQ358809	-	-	----	..	----	..
B.BR.02.02BR013.DQ358810	A	C	----	..	----	..
B.BR.03.BREPM1023.EF637057	-	-	----	..	----	..
B.BR.03.BREPM1024.EF637056	-	-	----	..	----	..
B.BR.03.BREPM1027.EF637054	-	-	----	..	----	..
B.BR.03.BREPM1028.EF637053	-	-	----	..	----	..
B.BR.03.BREPM1032.EF637051	-	-	----	..	----	..
B.BR.03.BREPM1033.EF637050	-	-	----	..	----	..
B.BR.03.BREPM1035.EF637049	-	-	----	..	----	..
B.BR.03.BREPM1038.EF637048	-	-	----	..	----	..
B.BR.03.BREPM1040.EF637047	-	-	----	..	----	..
B.BR.03.BREPM2012.EF637046	-	-	----	..	----	..
B.BR.04.BREPM1066.FJ195090	-	-	----	..	----	..
B.BR.04.BREPM1070.FJ195086	-	-	-----C-	..	-----G-	..
B.BR.05.BREPM1081.FJ195091	-	-	----	..	----	..
B.BR.05.BREPM1084.FJ195088	-	-	----	..	----	..
B.BR.05.BREPM1093.FJ195089	-	-	----	..	----	..
B.CA.96.WC10C_4.AY314056	-	-	----	..	----	..
B.CN.01.CNHN24.AY180905	-	-	----	..	----	..
B.CN.02.02HNsc11.DQ007903	-	-	----	..	----	..
B.CN.05.05CNHB_hp3.DQ990880	-	-	----	..	----	..
B.CN.x.RL42.U71182	-	-	----	..	----	..
B.CU.99.Cu19.AY586542	-	-	----	..	----	..
B.CU.99.Cu43.AY586543	-	-	----	..	----	..
B.DE.86.D31.U43096	-	-	----	..	----	..
B.DE.86.HAN.U43141	-	-	----	..	----	..
B.DK.01.CTL_016.EF514704	-	-	----	..	----	..
B.DK.01.CTL_017.EF514705	-	-	----	..	----	..
B.DK.01.CTL_018.EF514706	-	-	----	..	----	..
B.DK.01.CTL_023.EF514707	-	-	----	..	----	..
B.DK.01.CTL_030.EF514708	-	-	----	..	----	..
B.DK.01.CTL_033.EF514709	-	-	----	..	----	..
B.DK.01.CTL_035.EF514710	-	-	----	..	----	..
B.DK.01.CTL_043.EF514712	-	-	----	..	----	..
B.DK.04.PMVL_012.EF514699	-	-	----	..	----	..
B.DK.04.PMVL_013.EF514700	-	-	----	..	----	..
B.DK.04.PMVL_018.EF514697	-	-	----	..	----	..
B.DK.04.PMVL_025.EF514702	-	-	----	..	----	..
B.DK.04.PMVL_027.EF514698	-	-	----	..	----	..
B.DK.04.PMVL_039.EF514703	-	-	-----G	GBAU	GG	GGG-
B.DK.04.PMVL_049.EF514701	-	-	----	..	----	..
B.ES.05.ES.EU786672	-	-	----	..	----	..
B.ES.06.ES.EU786675	-	-	----	..	----	..
B.ES.06.ES.EU786676	-	-	----	..	----	..
B.ES.06.ES.EU786677	-	-	----	..	----	..
B.ES.07.ES.EU786678	-	-	----	..	----	..
B.ES.07.ES.EU786679	-	-	----	..	----	..
B.ES.07.ES.EU786680	-	-	----	..	----	..
B.ES.89.U61.DQ854716	-	-	----	..	----	..
B.GA.88.OYI.M26727	-	-	----	..	----	..
B.GB.83.CAM1.D10112	-	-	----	..	----	..
B.GB.86.GB8.AJ271445	-	-	-----A-G	A	----	..
B.GB.x.MANC.U23487	-	-	----	..	----	..
B.HK.x.HK003.FJ460500	-	-	----	..	----	..
B.HK.x.HK004.FJ460501	-	-	----	..	----	..
B.IN.x.11807.EF694037	-	-	----	..	----	..
B.IT.05.SG1.DQ672623	-	-	----	..	----	..
B.JP.00.DR2508.AB289588	-	-	----	..	----	..
B.JP.01.DR388.AB289590	-	-	----	..	----	..
B.JP.04.04JPDR6075B.AB221125	-	-	----	..	----	..

B.JP.04.DR6089.AB286955	-	-	----	..	----	..
B.JP.05.DR6538.AB287363	-	-	----	..	----	..
B.JP.05.DR6737.AB287364	-	-	----	..	----	..
B.JP.05.DR7060.AB287366	-	-	----	..	----	..
B.JP.05.DR7065.AB287368	-	-	----	..	----	..
B.JP.99.DR1348.AB287370	-	-	----	..	----	..
B.KR.04.04KMH5.DQ295193	-	-	----	..	----	..
B.KR.04.04LHS6.AY839827	-	-	----	..	----	..
B.KR.04.04LSK7.DQ295192	-	-	----	..	----	..
B.KR.04.04WK7_HIV_1_wk.DQ295194	-	-	----	..	----	..
B.KR.05.05CSR3.DQ837381	-	-	----	..	----	..
B.MM.99.mSTD101.AB097870	-	-	----	..	----	..
B.NL.00.671_00T36.AY423387	-	-	----	..	----	..
B.NL.86.3202A21.U34604	-	-	----	..	----	..
B.NL.96.H434_42_A1.AY970948	-	-	----	..	----	..
B.RU.04.04RU128005.AY682547	-	-	----	..	----	..
B.RU.04.04RU129005.AY751406	-	-	----	..	----	..
B.RU.04.04RU139089.AY751407	-	-	----	..	----	..
B.RU.04.04RU139095.AY819715	-	-	----	..	----	..
B.TH.90.BK132.AY173951	-	-	----	..	----	..
B.TW.94.TWCYS.AF086817	-	-	----	..	----	..
B.US.00.14294_1.DQ853436	-	-	----	..	----	..
B.US.00.ES1_20.EF363123	-	-	----	..	----	..
B.US.04.ES10_53.EF363127	-	-	----	..	----	..
B.US.04.ES4_24.EF363124	-	-	----	..	----	..
B.US.04.ES8_43.EF363126	-	-	----	..	----	..
B.US.83.5018_83.AY835777	-	-	----	..	----	..
B.US.83.5157_83.AY835781	-	-	----	..	----	..
B.US.83.RF.M17451	-	-	----	..	----	..
B.US.83.SF2.K02007	-	-	----	..	----	..
B.US.84.5019_84.AY835779	-	-	----	..	----	..
B.US.84.MNCG.M17449	-	-	----	..	----	..
B.US.84.NY5CG.M38431	-	-	----	..	----	..
B.US.84.SF33.AY352275	-	-	----	..	----	..
B.US.85.5077_85.AY835769	-	-	----	..	----	..
B.US.85.Ba_L.AB221005	-	-	----	..	----	..
B.US.86.5084_86.AY835775	-	-	----	..	----	..
B.US.86.5096_86.AY835749	-	-	----	..	----	..
B.US.86.5127_86.AY835774	-	-	----	..	----	..
B.US.86.AD87.AF004394	-	-	----	..	----	..
B.US.86.JRFL.U63632	-	-	----	..	----	..
B.US.86.YU_2.M93258	-	-	----	..	----	..
B.US.87.5113_87.AY835758	-	-	----	..	----	..
B.US.87.BC.L02317	-	-	----	..	----	..
B.US.88.5160_88.AY835763	-	-	----	..	----	..
B.US.88.WR27.AF286365	-	-	----	..	----	..
B.US.89.P896.U39362	-	-	----	..	----	..
B.US.90.US1.AY173952	-	-	----	..	----	..
B.US.90.US2.AY173953	-	-	----	..	----	..
B.US.90.US3.AY173954	-	-	----	..	----	..
B.US.90.US4.AY173955	-	-	----	..	----	..
B.US.90.WCIPR.U69591	-	-	----	..	----	..
B.US.90.WEAU160.U21135	-	-	----	..	----	..
B.US.91.5048_91.AY835761	-	-	----	..	----	..
B.US.91.DH12_3.AF069140	-	-	----	..	----	..
B.US.93.WCD32P0793.DQ487188	-	-	----	..	----	..
B.US.93.WCM32P0793.DQ487190	-	-	----	..	----	..
B.US.94.5082_94.AY835773	-	-	----	..	----	..
B.US.95.5073_95.AY835768	-	-	----	..	----	..
B.US.96.5155_96.AY835753	-	-	----	..	----	..
B.US.97.ARES2.AB078005	-	-	----	..	----	..
B.US.98.15384_1.DQ853463	-	-	----	..	----	..
B.US.98.98USHVTN1925c1.AY560107	-	-	----	..	----	..
B.US.98.98USHVTN3605c9.AY560108	-	-	----	..	----	..
B.US.98.98USHVTN8229c6.AY560109	-	-	-----A-G	A	----	..
B.US.98.98USHVTN941c1.AY560110	-	-	----	..	----	..
B.US.98.WC3_0498_4.EF175212	-	-	----	..	----	..
B.ZA.03.03ZAPS045MB2.DQ396398	-	-	----	..	----	..

D) The stretch of A in loop I (nt 301-305)

Accession	PLAI	AAAAA	UUUUGA
B.FR.83.HXB2	LAI	IIIB	BRU	K03455	-----
B.AU.86.MBC200	AF042100	-----	A.....U	-----
B.AU.87.MBC925	AF042101	-----	A.....	-----
B.AU.95.MBCC54	AF042103	-----U	-----
B.AU.96.MBCC98	AF042104	-----	-----
B.AU.96.MBCD36	AF042105	-----	-----
B.AU.x.1181	AF538302	-----UU	-----
B.AU.x.2870718	AY857022	G-----	-----
B.AU.x.8634991	AY857144	-----	AUAAAAA	-----
B.AU.x.9125091	AY857165	-----U	-----
B.AU.x.C24	AF538304	-----UA	-----
B.AU.x.C42	AF538305	-----	-----
B.AU.x.C76	AF538306	-----	-----
B.AU.x.C92	AF538307	-----	-----
B.AU.x.VH	AF146728	-----	-----
B.BR.02.02BR008	DQ358808	-ΔΔΔΔUU	-----
B.BR.02.02BR011	DQ358809	-----	C-UAAAA	-----
B.BR.02.02BR013	DQ358810	-----	UAAAA	-----
B.BR.03.BREPM1023	EF637057	-----U	-----
B.BR.03.BREPM1024	EF637056	-----	-----
B.BR.03.BREPM1027	EF637054	-ΔΔΔ	-----
B.BR.03.BREPM1028	EF637053	-----	-----
B.BR.03.BREPM1032	EF637051	-----	A.....U	-----
B.BR.03.BREPM1033	EF637050	-ΔΔΔΔUU	-----
B.BR.03.BREPM1035	EF637049	-ΔΔΔ	UUUUU	-----
B.BR.03.BREPM1038	EF637048	-----U	-----
B.BR.03.BREPM1040	EF637047	-ΔΔΔU	-----
B.BR.03.BREPM2012	EF637046	-----	A.....	-----
B.BR.04.BREPM1066	FJ195090	-----	-----
B.BR.04.BREPM1070	FJ195086	-----	-----
B.BR.05.BREPM1081	FJ195091	-----	AUUAAAAA	-----
B.BR.05.BREPM1084	FJ195088	-----	UAAAAUU	-----
B.BR.05.BREPM1093	FJ195089	-----U	-----
B.BR.89.BZ167	AY173956	-----U	-----
B.CA.96.WC10C	4.AY314056	-ΔΔU	-----
B.CA.97.CANB3FULL	AY779553	-----	-----
B.CA.x.HDM003V01	DQ322227	-----C	-----
B.CN.01.CNHN24	AY180905	-----UU	-----
B.CN.02.02HNsc11	DQ007903	-----	A.....UU	-G-----
B.CN.05.05CNHB	hp3.DQ990880	-ΔΔΔΔUU	-----
B.CN.x.RL42	U71182	-ΔΔΔ	Δ-----
B.CU.99.Cu19	AY586542	-----U	-----
B.CU.99.Cu43	AY586543	-----U	-----
B.DE.86.D31	U43096	-----	-----
B.DE.86.HAN	U43141	-----	-----
B.DK.01.CTL_016	EF514704	G-ΔUU	-G-----
B.DK.01.CTL_017	EF514705	G-U-Δ	-----
B.DK.01.CTL_018	EF514706	-ΔΔΔ	-----
B.DK.01.CTL_023	EF514707	-----	-----
B.DK.01.CTL_030	EF514708	-----	-----
B.DK.01.CTL_033	EF514709	-G-ΔU	-----
B.DK.01.CTL_035	EF514710	-ΔΔΔU	-----
B.DK.01.CTL_041	EF514711	-ΔΔU	-----
B.DK.01.CTL_043	EF514712	-----U	-----
B.DK.04.PMVL_012	EF514699	-U--	-----
B.DK.04.PMVL_013	EF514700	-ΔΔ	C-----
B.DK.04.PMVL_018	EF514697	-----U	-----
B.DK.04.PMVL_025	EF514702	-U--	-----
B.DK.04.PMVL_027	EF514698	C-----	A.....U	-----
B.DK.04.PMVL_039	EF514703	-----UU	-----
B.DK.04.PMVL_049	EF514701	-----U	-----
B.ES.05.ES	EU786672	-----	-----
B.ES.06.ES	EU786674	-ΔΔUU	-----
B.ES.06.ES	EU786675	-U--	-----
B.ES.06.ES	EU786676	-ΔΔΔ	-----
B.ES.06.ES	EU786677	-----	-----
B.ES.07.ES	EU786678	G----	-----
B.ES.07.ES	EU786679	-----	A.....UU	-----
B.ES.07.ES	EU786680	G----U	-----
B.ES.89.U61	DQ854716	-----	-----
B.GA.88.OYT	M26727	-----U	-----
B.GB.83.CAM1	D10112	-ΔΔΔΔ	UUUA	-----
B.GB.86.GB8	AJ271445	-----	-----
B.GB.x.MANC	U23487	-----	-----
B.HK.x.HK003	FJ460500	-----U	-----
B.HK.x.HK004	FJ460501	-----U	-----
B.IN.x.11807	EF694037	-----	-----
B.IT.05.SG1	DQ672623	-ΔΔUU	-----
B.JP.00.DR2508	AB289588	-ΔΔΔU	-----
B.JP.01.DR388	AB289590	-ΔΔC	-----
B.JP.04.04JPDR6075B	AB221125	-ΔΔΔU	-----
B.JP.04.DR6089	AB286955	G--ΔU	-----
B.JP.05.DR6538	AB287363	-----	A.....UU	-----
B.JP.05.DR6737	AB287364	-ΔΔUU	-----
B.JP.05.DR7060	AB287366	-----	UAAAAU	-----
B.JP.05.DR7065	AB287368	-ΔΔΔUU	-----
B.JP.99.DR1348	AB287370	-----C	-----
B.KR.04.04KMH5	DQ295193	-----U	-----
B.KR.04.04LHS6	AY839827	-ΔΔΔΔ	UUUU	G-----
B.KR.04.04LSK7	DQ295192	G--ΔΔUU	-----
B.KR.04.04WK7_HIV_1	wk.DQ295194	-----UU	-----
B.KR.05.05CSR3	DQ837381	-----U	-----
B.MM.99.mSTD101	AB097870	-----	-C-----
B.NL.00.671_00T36	AY423387	U----	A.....	-----
B.NL.86.3202A21	U34604	-----U	-----
B.NL.96.H434_42_A1	AY970948	N----	A.....	-----
B.RU.04.04RU128005	AY682547	-----	A.....	UUUU	-----
B.RU.04.04RU129005	AY751406	-----	-----
B.RU.04.04RU139089	AY751407	-----	UAAAAU	-C-----
B.RU.04.04RU139095	AY819715	-----U	-----
B.TH.90.BK132	AY173951	-----	-----
B.TW.94.TWCYS	AF086817	-----U	-----
B.US.00.14294_1	DQ853436	-U--	-----
B.US.00.ES1_20	EF363123	-ΔΔU	-----
B.US.04.ES10_53	EF363127	-----U	-----
B.US.04.ES4_24	EF363124	-----	-----
B.US.04.ES8_43	EF363126	G--ΔU	-----
B.US.83.5018_83	AY835777	-----	-----
B.US.83.5157_83	AY835781	-----	-----
B.US.83.RF.M17451		G----	-----
B.US.83.SF2.K02007		-ΔΔΔU	-----
B.US.84.5019_84	AY835779	-----	-----
B.US.84.MNCG	M17449	-----U	-C-----
B.US.84.NY5CG	M38431	-----	-----
B.US.84.SF33	AY352275	-----	A.....	-----
B.US.85.5077_85	AY835769	-----	-----
B.US.85.Ba_L	AB221005	-----U	-----
B.US.86.5084_86	AY835775	-----	-----
B.US.86.5096_86	AY835749	-ΔΔU	-----
B.US.86.5127_86	AY835774	-----	-----
B.US.86.AD87	AF004394	-----	-----
B.US.86.JRFL	U63632	-----	-----
B.US.86.YU_2	M93258	-----	A.....U	-----
B.US.87.5113_87	AY835758	-----	-----
B.US.87.BC	L02317	-----	-----
B.US.88.5160_88	AY835763	-----	-----
B.US.88.WR27	AF286365	-U--	-----
B.US.89.P896	U39362	-ΔΔUU	-----
B.US.90.US1	AY173952	-----	-----
B.US.90.US2	AY173953	-----U	-----
B.US.90.US3	AY173954	-ΔΔ	-----
B.US.90.US4	AY173955	-ΔΔU	-----
B.US.90.WCIPR	U69591	-----	-----
B.US.90.WEAU	U160.U21135	G--Δ	-----
B.US.91.5048_91	AY835761	-----	-----
B.US.91.DH12_3	AF069140	-----	-----
B.US.93.WCD32P0793	DQ487188	-----U	-----
B.US.93.WCM32P0793	DQ487190	-----	-----
B.US.94.5082_94	AY835773	-----	-----
B.US.95.5073_95	AY835768	-----	-----
B.US.96.5155_96	AY835753	-ΔΔU	-----
B.US.97.ARES2	AB078005	-----	-C-----
B.US.98.15384_1	DQ853463	-U--	-----
B.US.98.98USHVTN1925c1	AY560107	-----U	-----
B.US.98.98USHVTN3605c9	AY560108	-----U	-----
B.US.98.98USHVTN8229c6	AY560109	-----UU	-----
B.US.98.98USHVTN941c1	AY560110	-----U	-----
B.US.98.WC3_0498_4	EF175212	G----	-----
B.ZA.03.03ZAPS045MB2	DQ396398	-ΔΔU	-----