CHAPTER 4

Results

4.1 The results of HIV-1 V3 serotype

All samples were examined HIV-1 serotype by SSEIA that detect antibodies binding to the V3 gp120 region of HIV-1 subtypes B and E (CRF01_AE). The results of HIV-1 V3 serotype that separated by study population and year of sample collection show in Table 4.1.

Table 4.1 The results of HIV-1 V3 serotype in each study population that separated by year of sample collection

Study population	Year of collection	Subtype CRF01_AE	Subtype B	No sample fot detection	Indeterminate results	Total
1	2009	298	25	81		404
	2010	454	80	41	2	577
	2011	597	97	39		733
	2012	574	85	20	7	686
	Total	1,923	287	181	9	2,400
	%	80.13	11.96	7.54	0.38	100
2	2010	1				1
	2011	16	5			21
	2012	25	2			27
	2013	1				1
	Total	43	7			50
	%	86	14			100
3	2012	69	16			85
	2013	6	1			7
	Total	75	17			92
	%	81.52	18.48			100
Total		2,041	311	181	9	2,542
%		80.29	12.23	7.12	0.35	100

All study populations (2,542 patients) composed of V3 serotype subtype CRF01_AE 80.29% (2,041 patients), subtype B 12.23% (311 patients), no sample for detection 7.12% (181 patients), and indeterminate serotypes by SSEIA 0.35% (9 patients) that show in Figure 4.1.

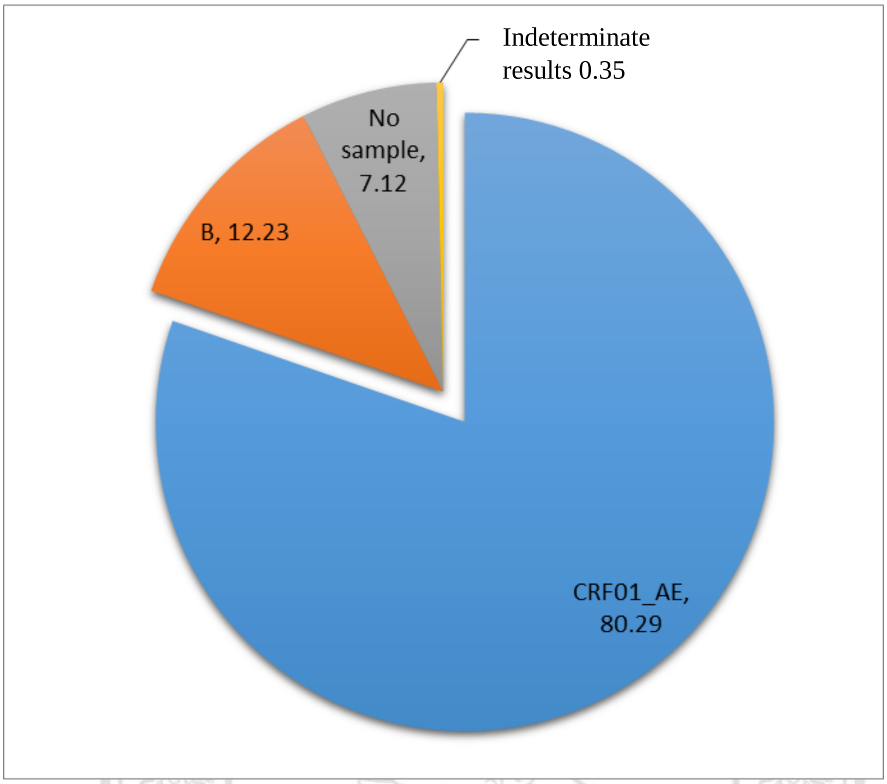


Figure 4.1 The distribution of HIV-1 V3 serotypes in all study populations

In the first study population (2,400 the treatment-experienced HIV-1 infected patients from Thai citizens) composed of V3 serotype subtype CRF01_AE 80.13% (1,923 patients), subtype B 11.96% (287 patients), no sample for detection 7.54% (181 patients), and indeterminate serotypes by SSEIA 0.38% (9 patients).

In the second study population (50 the treatment-experienced HIV-1 infected patients from non-Thai citizens) composed of V3 serotype subtype CRF01_AE 86.00% (43 patients), and subtype B 14.00% (7 patients).

In the third study population was naïve-treatment HIV-1 infected patients or newly diagnosed HIV patients. All patients were 92 patients that composed of V3 serotype subtype CRF01_AE 81.52% (75 patients), and subtype B 18.48% (17 patients).

The available samples were classified the HIV-1 V3 serotype that show in Table 4.2. During 2009 to 2013, V3 serotype CRF01_AE were about 92.26%, 84.73%, 85.73%, 85.86% and 87.50%, respectively, while subtype B accounted for 7.74%, 14.90%, 14.27%, 13.24% and 12.50%. The samples that showed indeterminate serotypes by SSEIA, were as 0.37%, and 0.9% in 2010 and 2012. This group was 9 samples which were 2 HIV-1 subtype C samples. The details of these samples show in Table 4.3. These samples presented high level of viral load and CD4 counts were range 142-517 cell/mm^3.

Table 4.2 The results of HIV-1 V3 serotype in the available samples of all study populations

Year of collection	CRF01_AE		Subtype B		Indeterminate results		Available sample
	No	%	No	%	No	%	
2009	298	92.26	25	7.74	0	0	323
2010	455	84.73	80	14.90	2	0.37	537
2011	613	85.73	102	14.27	0	0.00	715
2012	668	85.86	103	13.24	7	0.90	778
2013	7	87.50	1	12.50	0	0.00	8
Total	2041	86.45	311	13.17	9	0.38	2361

Table 4.3 The details of samples that showed indeterminate serotype

Study population	Year of collection	No	CMSU ID	*gag-pol* genotype	V3 serotype	CD4 (cells/mm^3)	Viral load (copies/ml)
1	2010	2	VIR003579	C	invalid	-	NA
			HDR693	CRF01_AE	invalid	290	5,130
	2012	7	VIR0031560	CRF01_AE	invalid	517	126,183
			VIR0031687	CRF01_AE	invalid	-	69,420
			VIR0031760	CRF01_AE	invalid	158	6,474
			VIR0031778	CRF01_AE	invalid	269	52,551
			VIR0031817	CRF01_AE	invalid	387	3,112
			VIR0031836	CRF01_AE	invalid	142	4,574
			VIR0031709	C	invalid	-	1,952,291
Total		9					

4.2 The results of *gag-pol* genotype

In the first and second populations, the nucleotide sequences of HIV-1 *gag-pol* genes from the routine drug resistance testing were analyzed by REGA HIV-1Subtyping Tool version 2.0 (http://dbpartners.stanford.edu/RegaSubtyping/), and Recombinant Identification Program: RIP (http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html). The results of HIV-1 *gag-pol* genotype show in Table 4.4, categorized by study populations and year of sample collection.

Table 4.4 The results of HIV-1 *gag-pol* genotype among the first and second study populations during 2009-2012

Study population	Year of collection	*gag-pol* genotype				Subtype characterizations of *gag-pol* sequences in IR					Total
		CRF01_AE	B	C	G	B/CRF01_AE	CRF01_AE/B	CRF01_AE/B/AE	B/CRF01_AE/B	B/C	
1	2009	394	8			1	1				404
	2010	558	14	1		1	1	1	1		577
	2011	707	24			1		1			733
	2012	652	24	2	1	5	2				686
	Total	2,311	70	3	1	8	4	2	1		2,400
	%	96.29%	2.92%	0.13%	0.04%	0.33%	0.17%	0.08%	0.04%		100%
2	2010	1									1
	2011	19		1						1	21
	2012	25	1				1				27
	2013	1									1
	Total	46	1	1			1			1	50
	%	92	2	2			2			2	100
Total		2,357	71	4	1	8	5	2	1	1	2,450
%		96.20%	2.90%	0.16%	0.04%	0.33%	0.20%	0.08%	0.04%	0.04%	100%

The HIV-1 *gag-pol* genotypes of the first and second study populations (2,450 the treatment-experienced HIV-1 infected patients from Thai and non-Thai citizens) were identified as HIV-1 subtype CRF01_AE 96.20% (2,357 patients), subtype B 2.90% (71 patients), subtype C 0.16% (4 patients), subtype G 0.04% (1 patient), and IR 0.69% (17 patients). The subtype characterizations of *gag-pol* sequences from IR were B/CRF01_AE 0.33% (8 patients), CRF01_AE/B 0.20% (5 patients), CRF01_AE/B/AE 0.08% (2 patients), B/CRF01_AE/B 0.04% (1 patient) and B/C recombination 0.04% (1 patient). The distribution of HIV-1 *gag-pol* genotypes among the first and second study populations show in Figure 4.2.

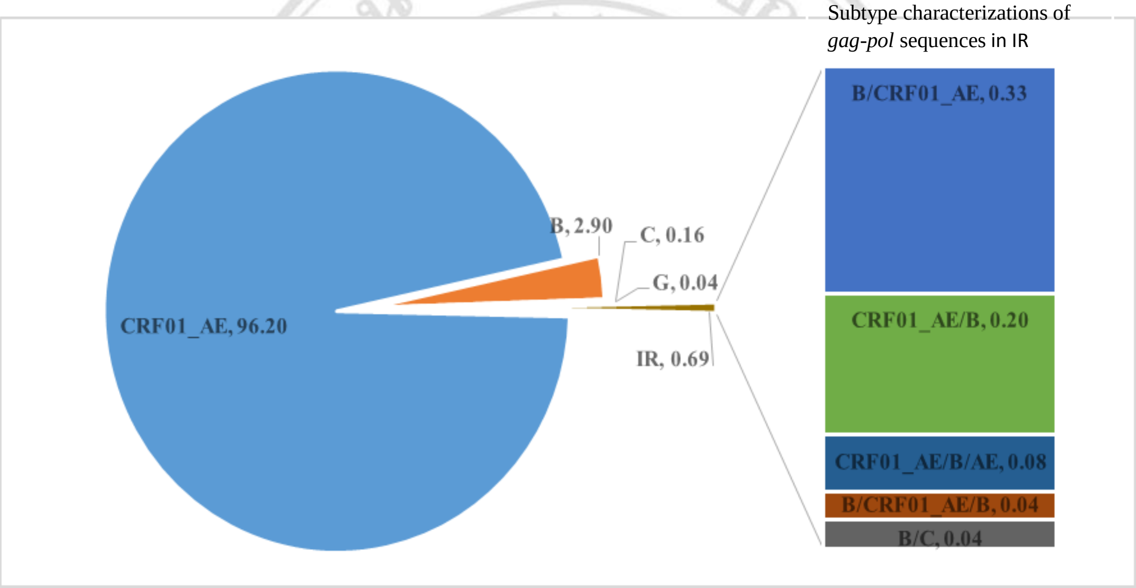


Figure 4.2 The distribution of HIV-1 *gag-pol* genotypes among the first and second study populations during 2009-2012

From the results of HIV-1 *gag-pol* genotype, the first study population (2,400 treatment-experienced HIV-1 infected patients who were Thai citizens) composed of patients who get infected with HIV-1 subtype CRF01_AE 96.29% (2,311 patients), subtype B 2.92% (70 patients), subtype C 0.13% (3 patients), subtype G 0.04% (1 patient), and inter-subtype recombination (IR) 0.63% (15 patients). Subtype characterizations of *gag-pol* sequences from HIV-1 inter-subtype recombinant isolates accounted for B/CRF01_AE 0.33% (8 patients), CRF01_AE/B 0.17% (4 patients), CRF01_AE/B/AE 0.08% (2 patients), and B/CRF01_AE/B recombination 0.04% (1 patient).

From the results of HIV-1 *gag-pol* genotype, the second study population (50 treatment-experienced HIV-1 infected patients who were non-Thai citizens) composed of patients who were infected with HIV-1 subtype CRF01_AE 92.00% (46 patients), subtype B 2.00% (1 patient), subtype C 2.00% (1 patient), and inter-subtype recombination (IR) 4.00% (2 patients) that the subtype characterizations of *gag-pol* sequences were reported as CRF01_AE/B 2.00% (1 patient) and B/C recombination 2.00% (1 patient).

The results of HIV-1 *gag-pol* genotype among the first and second study populations (2450 the treatment-experienced HIV-1 infected patients from Thai citizens and non-Thai citizens), categorized by year of sample collection show in Table 4.5.

Table 4.5 The results of HIV-1 *gag-pol* genotype in the the first and second study populations, categorized by year of sample collection

Year of collection	*gag-pol* genotype										Total
	CRF01_AE		Subtype B		Subtype C		Subtype G		IR		
	No	%	No	%	No	%	No	%	No	%	
2009	394	97.52	8	1.98	0	0	0	0	2	0.50	404
2010	559	96.71	14	2.42	1	0.17	0	0	4	0.69	578
2011	726	96.29	24	3.18	1	0.13	0	0	3	0.40	754
2012	677	94.95	25	3.51	2	0.28	1	0.14	8	1.12	713
2013	1	100.0									1
Total	2357	96.20	71	2.90	4	0.16	1	0.04	17	0.69	2450

In 2009, the *gag-pol* genotype of 404 HIV-1 infected patients in the first and second study populations were reported as HIV-1 subtype CRF01_AE 97.52% (394 patients), subtype B 1.98% (8 patients), and IR 0.50% (2 patients). Subtype characterizations of *pol* sequences from IR accounted for B/CRF01_AE 0.25% (1 patient), and CRF01_AE/B 0.25% (1 patient).

In 2010, the results of *gag-pol* genotype of 579 HIV-1 infected patients in the first and second study populations were HIV-1 subtype CRF01_AE 96.71% (559 patients), subtype B 2.42% (14 patients), subtype C 0.17% (1 patient) and IR 0.69% (4 patients). Subtype characterizations of *gag-pol* sequences from IR were demonstrated as B/CRF01_AE 0.17% (1 patient), CRF01_AE/B 0.17% (1 patient), CRF01_AE/B/AE 0.17% (1 patient), and B/CRF01_AE/B recombination 0.17% (1 patient).

In 2011, the gag-pol genotype of 754 HIV-1 infected patients of the first and second study populations were identified as HIV-1 subtype CRF01_AE 96.29% (726 patients), subtype B 3.18% (24 patients), subtype C 0.13% (1 patient) and IR 0.39% (3 patients). Subtype characterizations of *gag-pol* sequences from IR were determined as B/CRF01_AE 0.13% (1 patient), CRF01_AE/B/AE 0.13% (1 patient), and B/C recombination 0.13% (1 patient).

In 2012, the *gag-pol* genotypes of 713 HIV-1 infected patients of the first and second study populations were classified HIV-1 subtype CRF01_AE 94.95% (677 patients), subtype B 3.51% (25 patients), subtype C 0.28% (2 patients), subtype G 0.14% (1 patient) and IR 1.12% (8 patients). Subtype characterizations of *pol* sequences from IR were shown as B/CRF01_AE 0.70% (5 patients), and CRF01_AE/B 0.42% (3 patients).

During 2009 to 2012, the results of HIV-1 *gag-pol* genotype in the first and second study populations showed that HIV-1 subtype CRF01_AE infection accounted for 97.52%, 96.71%, 96.29%, and 94.95%, respectively. And the numbers of patients who infected with HIV-1 subtype B were gradually shown as 1.98%, 2.42%, 3.18%, and 3.51%, seqeuncely. The first HIV-1 subtype C infected patient was found in 2010, the second patient in 2011, and two patients in 2012. Moreover, one HIV-1 subtype G infected Thai citizen was found in 2012. Since 2009 to 2012, HIV-1 inter-subtype recombinant infected patients in the both study populations accounted for 0.5%, 0.69%, 0.4%, and 1.12%, respectively. All subtype characterizations of *gag-pol* sequences from HIV-1 IR (17 patients) composed of B/CRF01_AE 47% (8 patients), CRF01_AE/B 29% (5 patients), CRF01_AE/B/AE 12% (2 patient), B/CRF01_AE/B 6% (1 patients) and B/C recombination 6% (1 patient) that show in Figure 4.3.

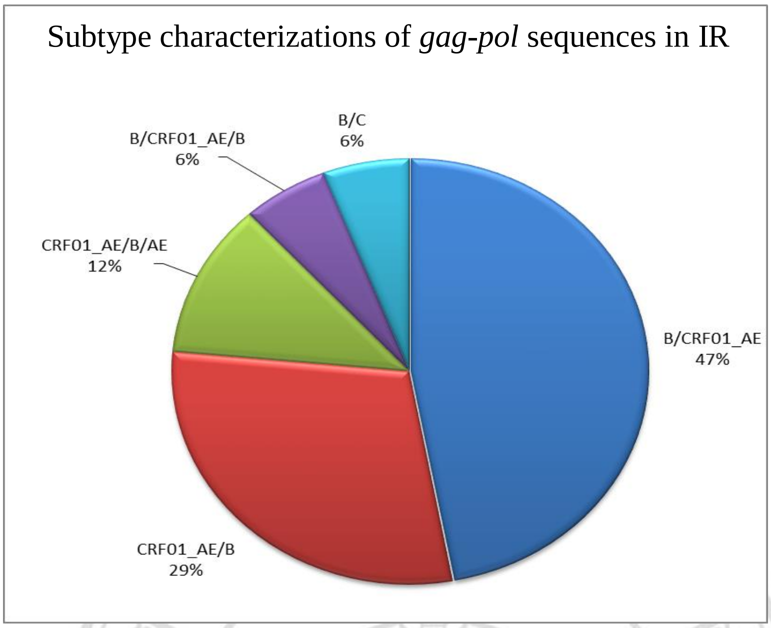


Figure 4.3 The subtype characterizations of *gag-pol* sequences from HIV-1 inter-subtype recombination

4.3 The results of evaluation of HIV-1 V3 serotype and *gag-pol* genotype

The results of HIV-1 V3 serotype and *gag-pol* genotype were compared for subtype conclusion. Total of study populations reported concordant results 81.76%, discordant results 9.88% and the others 8.36% that show in Table 4.6. The first study population (2400 the treatment-experienced HIV-1 infected patients from Thai citizens) showed 81.71% concordant results, 9.88% discordant results and the others (no sample, indeterminate serotype by SSEIA, subtype C, subtype G and IR) 8.41%. The second study population (50 the treatment-experienced HIV-1 infected patients from non-Thai citizens) showed 84.00% concordant results, 10.00% discordant results and the others (subtype C and IR) 6.00%.

Table 4.6 The results of evaluation of HIV-1 V3 serotype and *gag-pol* genotype in each study population and year of sample collection

Study population	Year of collection	Total	Concordant	Discordant	No sample	Indeterminate serotype	IR	Subtype C	Subtype G
1	2009	404	304	18	80		2		
	2010	577	464	67	40	1	4	1	
	2011	733	611	81	39		2		
	2012	686	582	71	17	6	7	2	1
	Total	2400	1961	237	176	7	15	3	1
	%	100%	81.71%	9.88%	7.33%	0.29%	0.63%	0.13%	0.04%
2	2010	1	1						
	2011	21	15	4			1	1	
	2012	27	25	1			1		
	2013	1	1						
	Total	50	42	5			2	1	
	%	100%	84.00%	10.00%			4.00%	2.00%	
Total		2450	2003	242	176	7	17	4	1
%		100%	81.76%	9.88%	7.18%	0.29%	0.69%	0.16%	0.04%

The concordant results from HIV-1 V3 serotype and *gag-pol* genotype were 81.76% (2003/2450 patients) that classified HIV-1 subtype CRF01_AE 79.51% (1948 patients) and subtype B 2.24% (55 patients) that show in Table 4.7.

Table 4.7 The results of evaluation of HIV-1 V3 serotype and *gag-pol* genotype that classified subtype

Result	Subtype		Conclusion subtype	Study population		Total	
	gag-pol	V3 serotype		1	2	No	%
Concordant Result	CRF01_AE	CRF01_AE	CRF01_AE	1907	41	1948	79.51%
	B	B	B	54	1	55	2.24%
	Total			1961	42	2003	81.76%
Discordant Result	CRF01_AE	B		229	5	234	9.55%
	B	CRF01_AE		8	0	8	0.33%
	Total			237	5	242	9.88%
Other				202	3	205	8.36%
Total				2400	50	2450	100%

In discordant results 9.88% (242 patients) composed of 9.55% (234 patients) that *gag -pol* genotype showed subtype CRF01_AE, while V3 serotype was identified subtype B and 0.33% (8 patients) had *gag -pol* genotype as subtype B, whereas V3 serotype was identified CRF01_AE.

We found that it showed fare agreement between HIV-1 V3 serotype and *gag-pol* genotype in the both populations. The Kappa coefficient were 0.30, 0.30 and 0.27 in the both populaions, the first and the second populations, respectively.

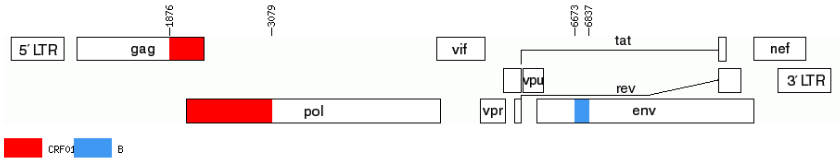
4.4 The results of HIV-1 *env* gp120 C2-V3-C3 genotype

In discordant results (241 patients), 73 RNA were HIV-1 *env* gp 120 C2-V3-C3 genotyping and analyzed by REGA HIV-1 Subtyping Tool version 2.0 http://dbpartners.stanford.edu/RegaSubtyping/, and Recombinant Identification Program: RIP http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html. These results show in Table 4.8.

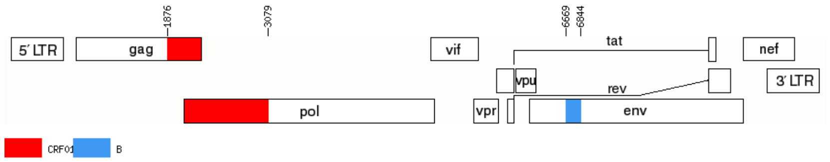
Table 4.8 The results of *gag-pol* genotype, V3 serotype and *env* C2V3C3 genotype in 73 discordant results

Case	*gag-pol* genotype	V3 serotype	*env* C2V3C3 genotype	No	Subtype
1	CRF01_AE	B	CRF01_AE	65 (89.04%)	CRF01_AE
2	B	E	B	1 (1.37%)	B
3	CRF01_AE	B	B	3 (4.11%)	CRF01_AE/B
4	B	E	CRF01_AE	4 (5.48%)	B/CRF01_AE
Total				73 (100%)	

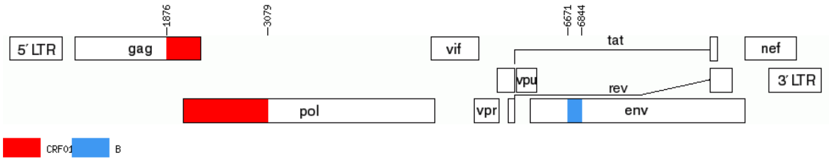
In 73 discordant results, subtype characterizations of *gag-pol* and *env* C2V3C3 sequences resulted subtype CRF01_AE about 65 patients (89.04%), subtype B 1 patient (1.37%), and HIV-1 inter-subtype recombination 7 patients (9.59%) that composed of 3 (4.11%) CRF01_AE/B recombinants of CRF01_AE subtype in *gag-pol* region and subtype B in *env* C2V3C3 region (VIR0031808, VIR0031722 and VIR0031037) that show genomes in Figure 4.4. We found these genomes had similar mosaic to CRF15_01B by estimate data on *gag-pol* and *env* C2V3C3 regions. While 4 (5.48%) B/CRF01_AE recombination between subtype B in *gag-pol* region and CRF01_AE in *env* C2V3C3 region (VIR0031718, VIR0031688, VIR003962 and VIR003963) that show genomes in Figure 4.5. We found these genomes were alike many CRFs, CRF48_01B or CRF51_01B or CRF54_01B by estimate datas on *gag-pol* and *env* C2V3C3 regions.



VIR0031808

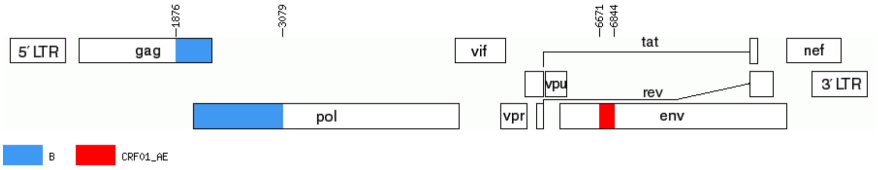


VIR0031722

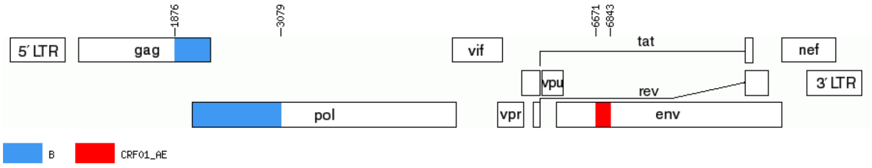


VIR0031037

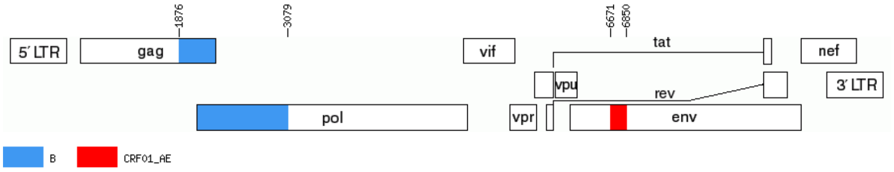
Figure 4.4 The three genomes of HIV-1 inter-subtype recombinant with CRF01_AE (red color) in *gag-pol* region and subtype B (blue color) in *env* C2V3C3 region



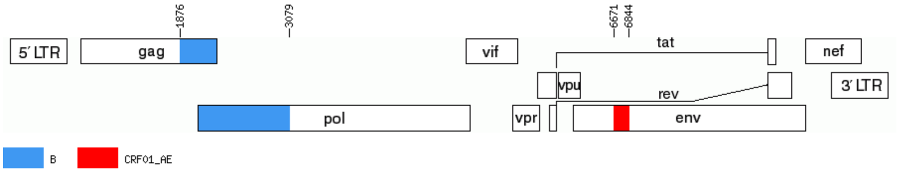
VIR0031718



VIR0031688



VIR003962



VIR003963

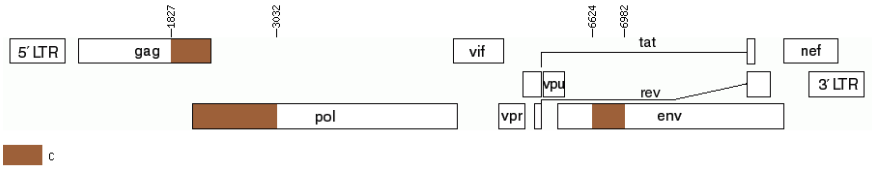
Figure 4.5 The four genomes of HIV-1 inter-subtype recombinant with subtype B (blue color) in *gag-pol* region and CRF01_AE (red color) in *env* C2V3C3 region

Then RNA of HIV-1 *gag-pol* genotype C, G and IR isolates were HIV-1 *env* gp 120 C2-V3-C3 genotyping. The details of these samples show in Table 4.9. Almost samples showed high level of HIV viral load, indicated that HIV is untreated and uncontrolled.

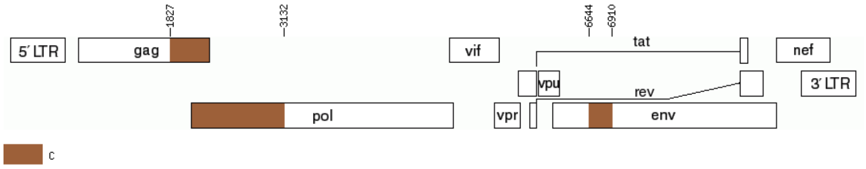
Table 4.9 The details of patients who infected with HIV-1 subtype C, G and inter-subtype recombinants

Study population	Year of collection	No	CMSU ID	*gag-pol* sequence	V3 serotype	CD4 (cells/mm^3)	Viral load (copies/ml)
1	2009	2	HDR454	CRF01_AE/B	E	NA	NA
			HDR370	B/CRF01_AE		280	NA
	2010	5	VIR003604	CRF01_AE/B	E	521	4,571
			VIR003514	CRF01_AE/B/AE	B	211	710,000
			HDR804	B/CRF01_AE	E	NA	NA
			HDR875	B/CRF01_AE/B		NA	866,236
			VIR003579	C	invalid	-	NA
	2011	2	VIR0031053	CRF01_AE/B/AE	E	143	2,486
			VIR0031143	B/CRF01_AE	E	NA	2,470,622
	2012	10	VIR0031720	CRF01_AE/B	E	285	8,081,342
			VIR0031867	CRF01_AE/B			553,539
			VIR0031667	B/CRF01_AE	B	97	17,104
			VIR0031704	B/CRF01_AE	E	2	371,420
			VIR0031706	B/CRF01_AE	B	95	72,599
			VIR0031765	B/CRF01_AE		120	289,412
			VIR0031781	B/CRF01_AE		1736	71,489
			VIR0031954	C	E		24,984
			VIR0031709	C	invalid		1,952,291
			VIR0031502	G	B	65	531,742
	Total	19					
2	2011	2	NAPHA020	C	E		11,800
			NAPHA024	B/C	B		239,000
	2012	1	NAPHA045	CRF01_AE/B	E		164,000
	Total	3					
Total		22					

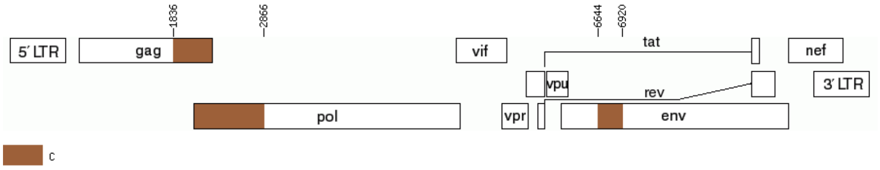
We found 3 HIV-1 infected patients that *gag-pol* and *env* C2V3C3 genotype identified subtype C (VIR003579, VIR0031709 and NAPHA020) who 2 Thai citizens and 1 non-Thai citizen. While VIR0031954 was identified C/CRF01_AE subtype because of *gag-pol* genotye showed subtype C and *env* C2V3C3 genotype was CRF01_AE. These genomes show in Figure 4.6. We could not conclude there were 3 pure subtype C and subtype C/CRF01_AE recombination until analyze near-full-genome sequencing. The genome of subtype C/CRF01_AE recombination (VIR0031954) had not similar breakpoint recombination data with currently 72 CRFs that have been engaged in the HIV database at Los Alamos National Laboratory. However, HIV-1 subtype C/CRF01_AE recombination had been detected in Thailand from other studies (Watanaveeradej *et al*., 2006 and Praparattanapan *et al*., 2011).



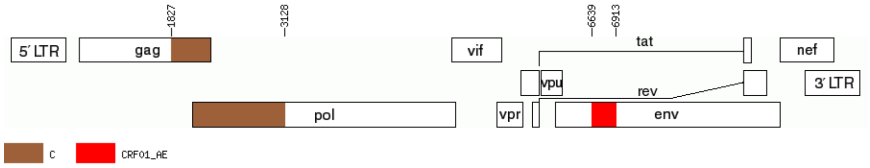
VIR003579



VIR0031709



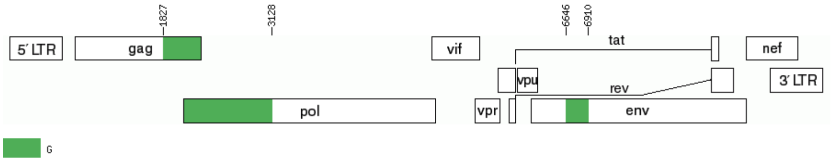
NAPHA020



VIR0031954

Figure 4.6 The genomes of HIV-1 subtype C and C recombinant with subtype CRF01_AE. Subtype C and CRF01_AE genomes appear as brown and red colored regions in the map, respectively.

One isolate was identified HIV-1 subtype G (VIR0031502) in the both regions, *gag-pol* and *env* C2V3C3 sequences that show in Figure 4.7.



VIR0031502

Figure 4.7 The genome of HIV-1 subtype G. The subtypes G compose genome appear as green colored regions in the map.

Eight samples had subtype characterizations of *gag-pol* sequences as B/CRF01_AE recombination. While six of eight isolats were found *env* C2-V3-C3 genotype as CRF01_AE subtype (HDR370, HDR804, VIR0031143, VIR0031667, VIR0031704, and VIR0031765), one of eight isolate resulted subtype B (VIR0031706). The other was not available sequencing (VIR0031781). We found the six genomes had similar breakpoint recombination to CRF58_01B by estimate data on *gag-pol* and *env* C2-V3-C3 regions. All genomes show the recombinant breakpoint data in Figure 4.8.

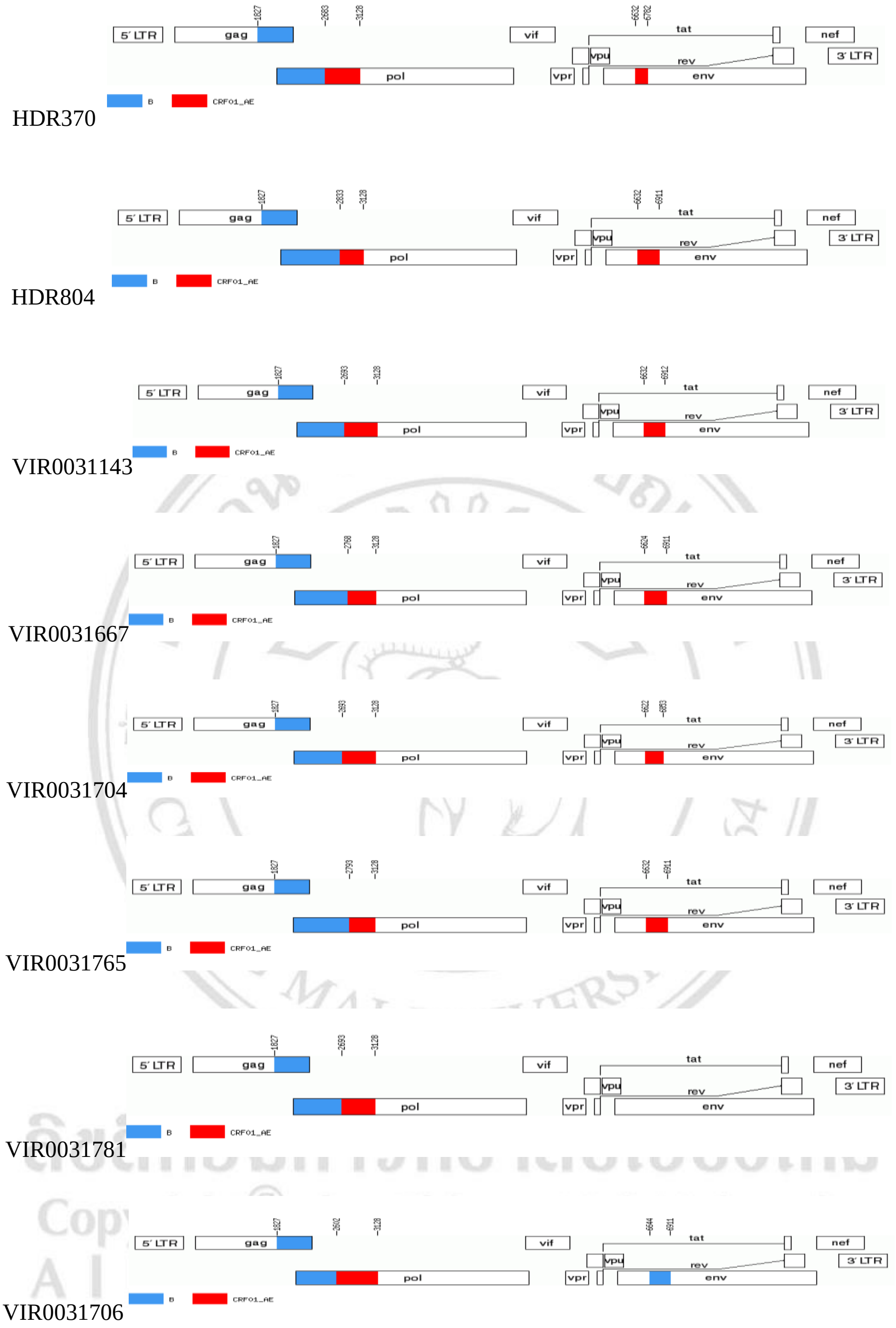


Figure 4.8 The eight genomes of HIV-1 infected patients who had subtype characterizations of *gag-pol* sequences as B/CRF01_AE. The subtype B and CRF01_AE genome appear as blue and red colored regions in the map, respectively. Six of eight genomes show CRF01_AE subtype on *env* C2-V3-C3 genome. Moreover one genome has *env* C2-V3-C3 genotype as B subtype.

Five samples which subtype characterizations of *gag-pol* sequences as CRF01_AE/B recombination. Two samples had *env* C2-V3-C3 genotype CRF01_AE (HDR454 and VIR0031720), while 3 samples were not available sequencing (VIR0031867, VIR003604 and NAPHA045). All genomes show the recombinant breakpoint data in Fgure 4.9. From the genome of *gag-pol* and *env* C2-V3-C3, we found VIR0031867 and VIR003604 had similar breakpoint recombination to CRF52_01B, while VIR0031720 had similar breakpoint recombination to CRF34_01B, by approximately.

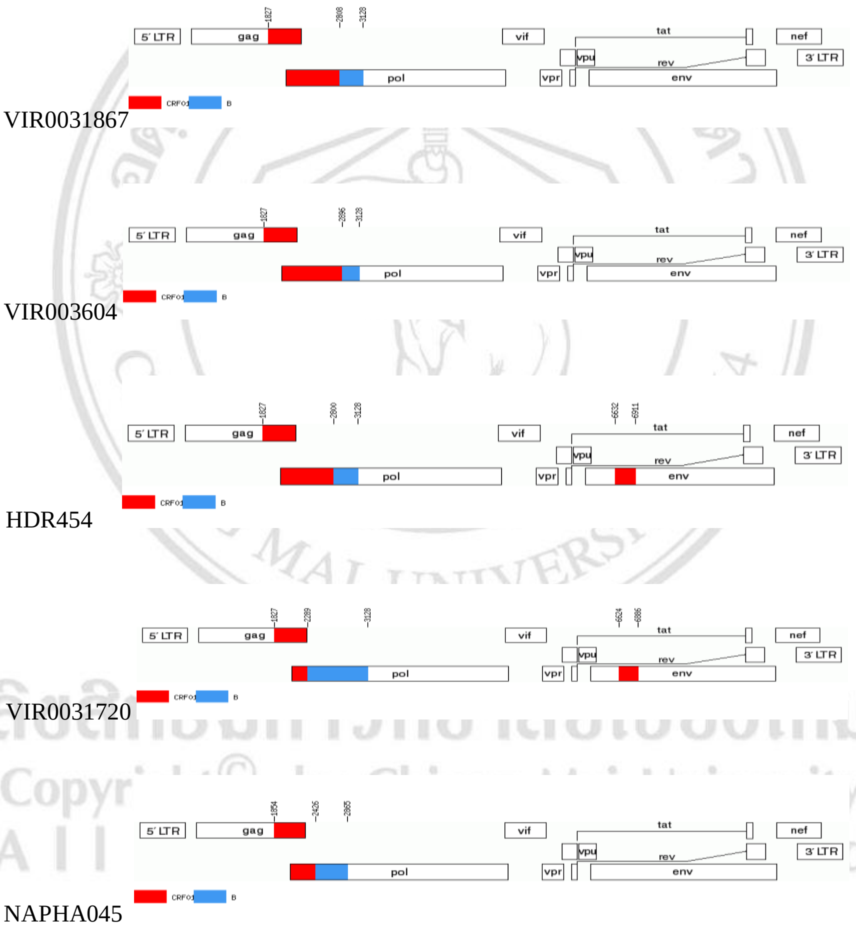
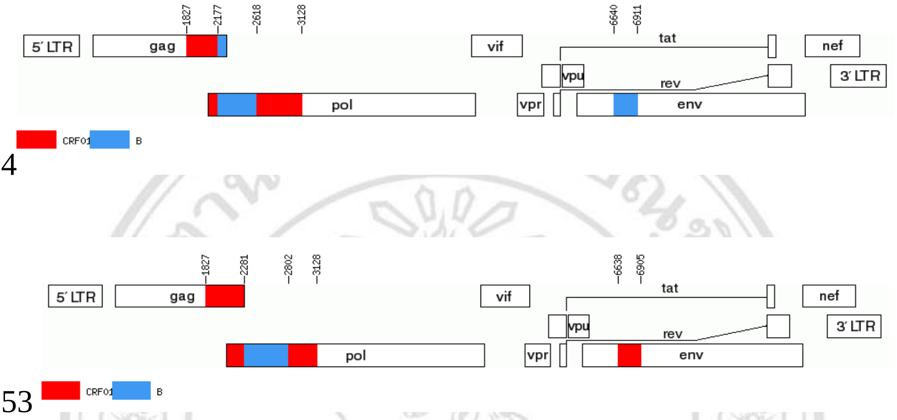


Figure 4.9 The five genomes of HIV-1 infected patients that subtype characterizations of *gag-pol* sequences show CRF01_AE/B. CRF01_AE and subtype B genome appear as red and blue colored regions in the map, respectively. Two genomes have *env* C2-V3-C3 genotype CRF01_AE.

Two samples which subtype characterizations of *gag-pol* sequences as CRF01_AE/B/AE recombination that *env* C2-V3-C3 genotypes were as subtype B (VIR003514), and CRF01_AE (VIR0031053), respectively. All genomes show the recombinant breakpoint data in Figure 4.10. VIR0031053 showed nearby breakpoint recombination to CRF34_01B.

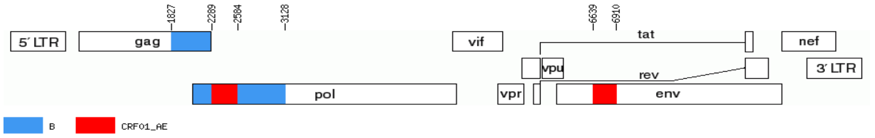


VIR003514

VIR0031053

Figure 4.10 The genomes of two samples of HIV-1 infected patients with subtype characterizations of *gag-pol* sequences show CRF01_AE/B/AE recombination. The CRF01_AE and B subtypes that compose genome appear as red and blue colored regions in the map, respectively. The genomes have *env* C2-V3-C3 genotype B and CRF01_AE subtype, respectively.

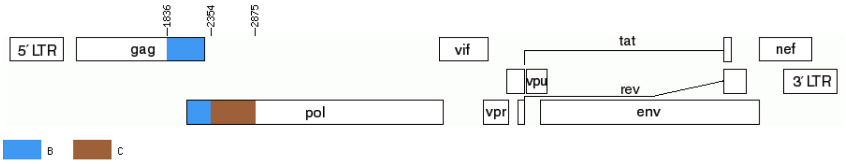
HDR875 was classified inter-subtype recombination by *gag-pol* sequence showed subtype characterizations as B/CRF01_AE/B and *env* C2-V3-C3 genotype as CRF01_AE subtype. The genome show the recombinant breakpoint data in Figure 4.11. HDR875 had similar breakpoint recombination to CRF33_01B by estimate.



HDR875

Figure 4.11 The genome of HIV-1 inter-subtype recombination which subtype characterizations of *gag-pol* sequence show B/CRF01_AE/B and *env* C2-V3-C3 genotype as CRF01_AE. The B subtypes and CRF01_AE that compose genome appear as blue and red colored regions in the map, respectively.

NAPHA024 was classified subtype B/C recombination by subtype characterizations of *gag-pol* sequence, but not available sequence by *env* C2-V3-C3 genotyping. This recombinant breakpoint data on *gag-pol* genome was estimate to similar with CRF07_BC that show in Figure 4.12.



NAPHA024

Figure 4.12 The genome of HIV-1 inter-subtype recombination which subtype characterizations of *gag-pol* sequence show B/C recombination. The B and C subtypes that compose genome appear as blue and brown colored regions in the map, respectively.

All subtype characterizations of *gag-pol* and *env* C2V3C3 sequences in the patients who infected with HIV-1 subtype C, G and inter-subtype recombinants are shown in Table 4.10.

Table 4.10 Subtype characterizations of *gag-pol* and *env* C2V3C3 sequences in the patients who infected with HIV-1 subtype C, G and inter-subtype recombinants

No	Sample ID	*gag-pol* sequence	*env* C2V3C3 sequence
1	VIR003579	C	C
2	VIR0031709	C	C
3	NAPHA020	C	C
4	VIR0031954	C	CRF01_AE
5	VIR0031502	G	G
6	VIR0031808	CRF01_AE	B
7	VIR0031722	CRF01_AE	B
8	VIR0031037	CRF01_AE	B
9	VIR0031718	B	CRF01_AE
10	VIR0031688	B	CRF01_AE
11	VIR003962	B	CRF01_AE
12	VIR003963	B	CRF01_AE
13	HDR370	B/CRF01_AE	CRF01_AE
14	HDR804	B/CRF01_AE	CRF01_AE
15	VIR0031143	B/CRF01_AE	CRF01_AE
16	VIR0031667	B/CRF01_AE	CRF01_AE
17	VIR0031704	B/CRF01_AE	CRF01_AE
18	VIR0031765	B/CRF01_AE	CRF01_AE
19	VIR0031781	B/CRF01_AE	Not Available
20	VIR0031706	B/CRF01_AE	B
21	HDR454	CRF01_AE/B	CRF01_AE
22	VIR0031867	CRF01_AE/B	Not Available
23	VIR003604	CRF01_AE/B	Not Available
24	VIR0031720	CRF01_AE/B	CRF01_AE
25	NAPHA045	CRF01_AE/B	Not Available
26	VIR003514	CRF01_AE/B/AE	B
27	VIR0031053	CRF01_AE/B/AE	CRF01_AE
28	HDR875	B/CRF01_AE/B	CRF01_AE
29	NAPHA024	B/C	B

4.5 The results of phylogenetic analysis

Seven samples that showed indeterminate serotypes by SSEIA and were subtype CRF01_AE strain by *gag-pol* genotyping, could not be genotyped in *env* gp120 C2-V3-C3 region, because the limitation of the samples. Subtypes were assessed by using Neighbor-joining tree of the HIV-1 *gag-pol* nucleotide sequences with the reference subtype which show the results in Figure 4.13. On the principle of phylogenetic analysis, the patients harboured the CRF01_AE virus, CRF15_AE and the inter-subtype recombinants composed of VIR0031760 and VIR0031817 isolates clustered with Ref.01_AB.CN.05.05GX001.GU564221 (In figure 4.13 that show in red circle). VIR0031687 isolate clustered with Ref.15_01B.TH.99.99TH_MU2079.AF516184 (In figure 4.13 that show in green circle). VIR0031836 isolate clustered between two CRFs, CRF01_AE, Ref.01_AE.AF.07.569M.GQ477441 and CRF22_01A such as Ref.22 01A1.CM.01.01CM 0001BBY.AY371159, respectively (In figure 4.13 that show in orange circle). VIR0031778 and VIR0031560 isolates branched off close to the root between two subtypes, subtype A1 and CRF22_01A such as Ref.A1.AU.03.PS1044Day0.DQ676872 and Ref.22_01A1.CM.02.02CM_3097MN.GQ229529, respectively. (In figure 4.13 that show in purple circle).

We analyzed the sequences of *gag-pol* and *env* regions of subtype C, subtype G and HIV-1 inter-subtype recombination isolates with 158 subtype reference sequences by construction of phylogenetic trees using the neighbour-joining method. The results of Neighbor-joining tree of the HIV-1 *gag-pol* and *env* nucleotide sequences in Figure 4.13 and 4.14, correspondingly.

Three patients infected with subtype C (VIR003579, NAPHA020, and VIR0031709), the *gag-pol* sequences were assessed subtype by phylogetic analysis showed harboured the subtype C virus that clustered with Ref.C.ZA.04.04ZASK146.AY772699 and Ref.C.IN.95.95IN21068.AF067155 (In figure 4.13 that show in yellow box). In contrast, we found the *env* genome of VIR0031709 clustered with Ref.38_BF1.UY.04.UY04_4022.FJ213782, and VIR003579 isolate branched off close to the root between two clusters, Ref.C.IN.95.95IN21068.AF067155 and Ref.17_BF.PE.02.PE02_PCR0155.EU581828, there were chance that they were recombinants (In figure 4.14 that show in yellow box).

VIR0031954 isolate was identified HIV-1 subtype C/CRF01_AE recombinant by *gag-pol* and *env* genotyping, and the results of the phylogenetic analysis showed a mosaic of subtypes C and CRF33_01B which clustered with Ref.C.IN.95.95IN21068.AF067155 in *gag-pol* region (In figure 4.13 that show in yellow box), and Ref.33_01B.ID.07.JKT194_C.AB547464 in *env* region (In figure 4.14 that show in orange box).

Although VIR0031502 was classified the patient infected with HIV-1 subtype G by *gag-pol* and *env* genotyping, from the phylogenetic tree this isolate clustered with Ref.43_02G.SA.03.J11456.EU697909 in *pol* region (In figure 4.13 that show in green box) and Ref.26_AU.CD.02.02CD KS069.FM877780 in *env* region (In figure 4.14 that show in green box), there was a chance that it was a recombinant.

NAPHA024 isolate that was identified HIV-1 subtype B/C recombinant by *gag-pol* genotyping and the phylogenetic tree showed it clustered with Ref. 08_BC.CN.06.nx2.HM067748 in *pol* region (In figure 4.13 that show in red box).

The samples of HIV-1 subtype B/CRF01_AE recombinants showed the complex inter-subtype recombination by phylogenetic analysis. The *gag-pol* sequences of these group showed harboured the various subtypes, for example subtype B, CRF01_AE, CRF15_AE, CRF03_AE, CRF33_AE, CRF34_AE or recombination subtypes. Whereas, we found the *env* genomes clustered with diverse inter-subtype recombinants such as CRF01_AE, CRF33_AE, CRF34_AE, CRF04_cpx, CRF26_AU or CRF28_BF.

The details of each isolate show in figure 4.13 and 4.14. VIR003963 and VIR003962 clustered with Ref.B.FR.83.HXB2 LAI IIIB BRU.K03455 in *pol* region (In figure 4.13 that show in dark blue box). In *pol* region VIR0031718 clustered with subtype B, Ref. B. NL 00.671 00T36.AY 423387 (In figure 4.13 that show in dark blue box). In *env* region, VIR0031718 clustered with subtype Ref.34_01B.TH.99.OUR2478P.EF165541 (In figure 4.13 that show in light blue box).

VIR0031688 isolate clustered with subtype B and Ref. B. US. 98. 1058 11.AY 331295, respectively in *pol* region (In figure 4.13 that show in dark blue box) and clustered with Ref.33_01B.MY.05.05MYKL007_1.DQ366659 in *env* region (In figure 4.14 that show in dark blue box). In *pol* region, VIR0031808, VIR003604, and VIR0031722 isolates clustered with Ref.15_01B.TH.96.M169.DQ354120 (In figure 4.13 that show in gray box). In *env* region, VIR0031808 clustered with subtype Ref.26_AU.CD.02.02CD_MBTB047.FM877782, and VIR0031722 clustered with Ref.28_BF.BR.99.BREPM12817.DQ085874 (In figure 4.14 that show in gray box). VIR0031037 clustered with Ref.01_AB.CN.05.05GX001.GU564221 in *pol* region (In figure 4.13 that show in red circle as same as VIR0031760 and VIR0031817).

While VIR0031667, VIR0031765, HDR875, and VIR0031720 isolates clustered with Ref.03 AB.RU.97.KAL153_2.AF193276 in *pol* region (In figure 4.13 that show in dark red box). In *env* region, VIR0031667 clustered with Ref.26_AU.CD.02.02CD KS069.FM877780 (In figure 4.14 that show in green box). VIR0031765 clustered with subtype Ref.34_01B.TH.99.OUR2478P.EF165541 (In figure 4.13 that show in light blue box). HDR875 and VIR0031720 clustered with Ref.33_01B.MY.05.05MYKL045_1.DQ366662 in *env* region (In figure 4.14 that show in dark red box). While HDR804, VIR0031143, VIR0031781, VIR0031704, HDR370 and VIR0031706 isolates branched off close to the root between two CRFs Ref.21_A2D.KE.91.KNH1254.AY945737 and Ref 09_cpx in *pol* region (In figure 4.13 that show in brown box). In *env* region, VIR0031704 and HDR370 clustered with Ref.01_AE.CN.05.05GX001.GU564221 (In figure 4.14 that show in brown box). VIR0031706 clustered with Ref.G.KE.93.HH8793_12_1.AF061641 in *env* region (In figure 4.14 that show in black box). VIR0031143 isolate clustered with subtype Ref.33_01B.MY.05.05MYKL045_1.DQ366662 in *env* region (In figure 4.14 that show in dark red box). NAPHA045 clustered with Ref.34 01B.TH.99.OUR2478P.EF165541 in *pol* region (In figure 4.13 that show in purple box).

In *pol* region, VIR003514 isolate clustered with Ref.33_01B.MY.05.05MYKL045_1.DQ366662, while HDR454, VIR0031053, and VIR0031867 isolates branched off close to the root between two subtypes, CRF33_01B (such as Ref.33 01B.MY.05.05MYKL045_1.DQ366662) and subtype A1, there were chance that they were recombinants (In figure 4.13 that show in orange box). In *env* region, VIR003514 clustered with Ref.04_cpx.GR.97.GR84_97PVMY.AF119819 (In figure 4.13 that show in purple box). HDR454 isolate clustered with Ref.01_AE.CN.05.05GX001.GU564221 in *env* region (In figure 4.14 that show in brown box). VIR0031867 clustered with Ref.33_01B.MY.05.05MYKL045_1.DQ366662 in *env* region (In figure 4.14 that show in dark red box).

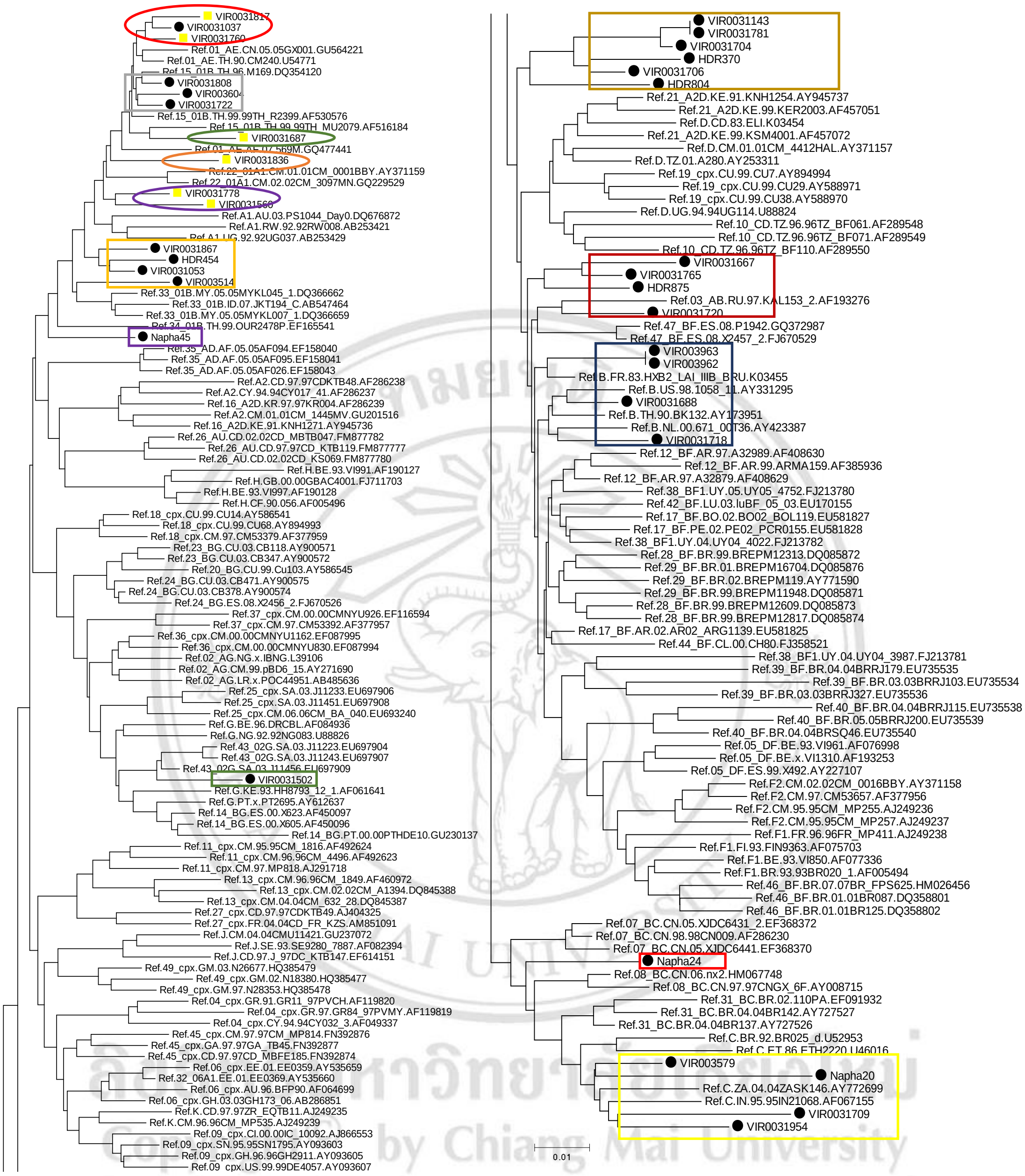


Figure 4.13 Neighbor-joining tree of the HIV-1 *pol* nucleotide sequences from the inter-subtype recombinants, subtype C, subtype G and indeterminate serotypes by SSEIA

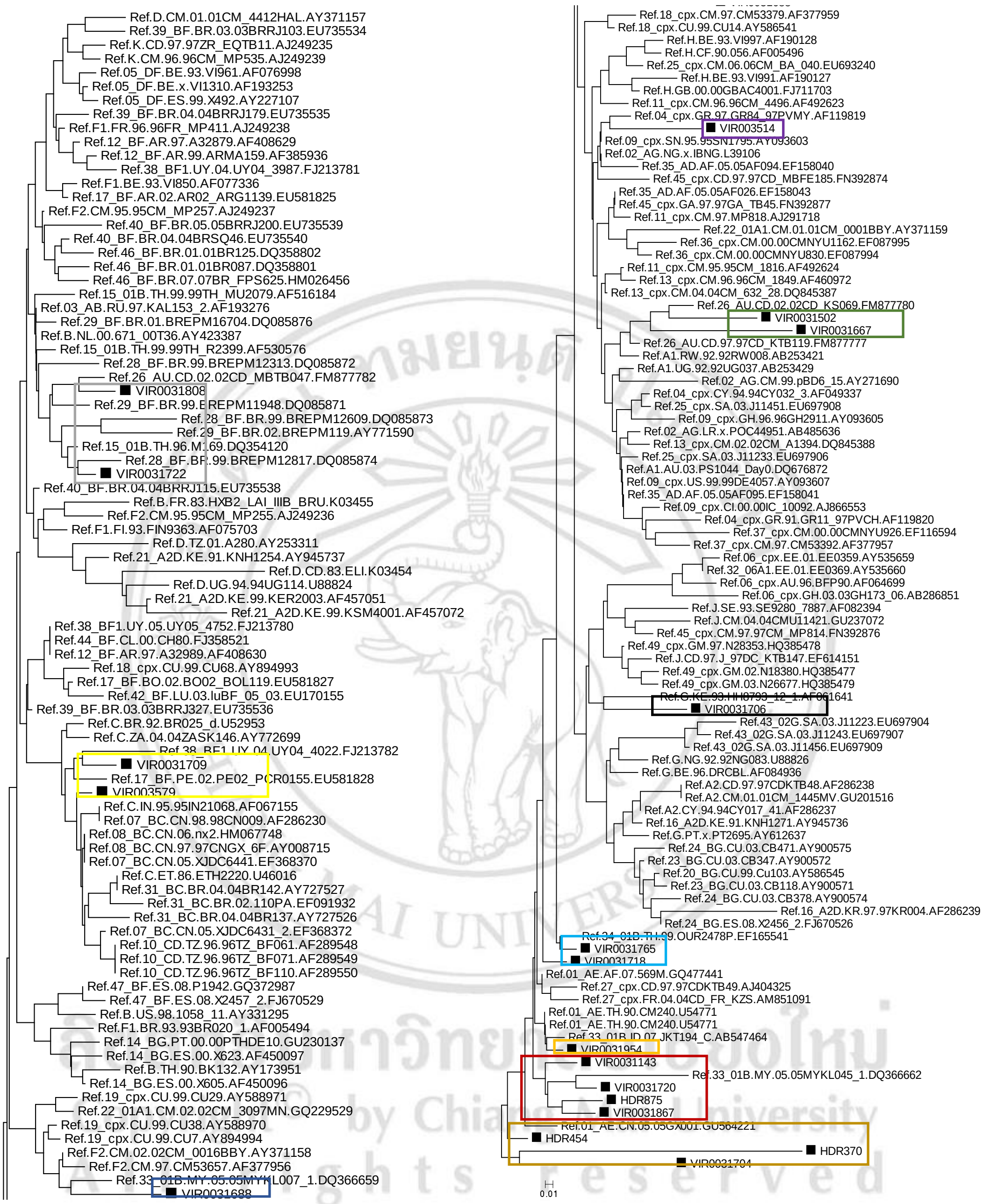


Figure 4.14 Neighbor-joining tree of the HIV-1 *env* nucleotide sequences from the inter-subtype recombination, subtype C and G

We concluded the HIV-1 subtypes in each study population that show in Table 4.11.

Table 4.11 The conclusion results of HIV-1 subtype in each study population

Study population	Subtype					Total	Inter-subtype recombination		
	CRF01_AE	B	C	G	IR		CRF01_AE/B	B/C	C/CRF01_AE
1	2308	66	2	1	23	2400	22		1
	96.17%	2.75%	0.08%	0.04%	0.96%	100%	0.92%		0.04%
2	46	1	1		2	50	1	1	
	92.00%	2.00%	2.00%		4.00%	100%	2.00%	2.00%	
3	75	17				92			
	81.52%	18.48%				100%			
Total	2429	84	3	1	25	2542	23	1	1
	95.55%	3.30%	0.12%	0.04%	0.98%	100%	0.90%	0.04%	0.04%

All study popultions, 2,542 HIV-1 infected patients, we could conclude that HIV-1 subtype CRF01_AE about 95.55% (2,429 patients), subtype B 3.30% (84 patients), subtype C 0.12% (3 patients), subtype G 0.04% (1 patient), and HIV-1 intersubtype recombination 0.98% (25 patients). Twenty-five HIV-1 intersubtype recombination infected patients composed of 23 HIV-1 recombinants of subtype B and CRF01_AE. One B/C intersubtype recombinant. We found one patient infected with HIV-1 subtype C/CRF01_AE recombinant.

In the first study population (2,400 treatment-experienced HIV-1 infected patients in Northern Thai citizens who participated in the National AIDS Program (NAP) by National Health Security Office of Thailand (NHSO) accounted for subtype CRF01_AE 2,308 patients (96.17%), subtype B 66 patients (2.75%), subtype C 2 patients (0.08%), subtype G 1 patient (0.04%), and HIV-1 intersubtype recombination 23 patients (0.96%). Twenty-three HIV-1 intersubtype recombination infected patients composed of 1 patient subtype C/CRF01_AE (0.04%), and 22 HIV-1 recombinants of subtype B and CRF01_AE (0.92%).

In the second study population, 50 treatment-experienced HIV-1 infected patients from Northern Thai citizens, who participated in the National Access to

Antiretroviral Program for People living with HIV/AIDS or NAPHA EXTENSION program by Global Fund AIDS Care. We concluded that HIV-1 subtype CRF01_AE about 46 patients (92%) subtype B 1 patient (2%), subtype C 1 patient (2%), and HIV-1 intersubtype recombination 2 patients (4%) that composed of 1 patient subtype CRF01_AE/B (2%), and 1 HIV-1 recombinant of subtype B and C (2%).

In the third study population, 92 naïve-treatment HIV-1 infected patients or newly diagnosed HIV patients who were blood donors in Northen Thailand. We could identified subtype CRF01_AE 75 patients (81.52%) and subtype B 17 patients (18.48%) by SSEIA.

This study was to investigate HIV-1 subtype distribution and intersubtype recombinants circulating in Northern Thailand that compose of Thai citizens and non-Thai citizens in order to monitoring the evolution of HIV-1 diversity before Thailand's participation in AEC. In Thai citizens that including the first and third study popultions, we concluded that HIV-1 subtype CRF01_AE was mainly subtype in Thai citizens (95.63%) follow by subtype B (3.33%), subtype C 2 patients (0.08%), subtype G 1 patient (0.04%), and HIV-1 intersubtype recombination 23 patients (0.92%). Twenty-three HIV-1 intersubtype recombination infected patients composed of 1 patient subtype C/CRF01_AE (0.04%), and 22 HIV-1 recombinants of subtype B and CRF01_AE (0.88%). In non Thai citizens, the second study population, We concluded that HIV-1 subtype CRF01_AE about 46 patients (92%) subtype B 1 patient (2%), subtype C 1 patient (2%), and HIV-1 intersubtype recombination 2 patients (4%) that compose of 1 patient subtype CRF01_AE/B (2%), and 1 HIV-1 recombinant of subtype B and C (2%). However, no significant difference of HIV-1 CRF01_AE subtype was mainly strain in HIV-1 infected patients between Thai citizens and non-Thai citizens and followed by subtype (*p*> 0.05).

The distribution of HIV-1 subtypes in HIV-1 treatment-experienced patients (the first and second study populations) HIV-1 subtype CRF01_AE was mainly subtype in HIV-1 treatment-experienced patients (96.08%) follow by subtype B (2.73%), subtype C 3 patients (0.12%), subtype G 1 patient (0.04%), and HIV-1 intersubtype recombination 25 patients (1.02%). Twenty-five HIV-1 intersubtype recombination infected patients composed of 1 patient subtype C/CRF01_AE (0.04%), 1 patient subtype B/C (0.04%), and 23 HIV-1 recombinants of subtype B and CRF01_AE (0.94%). In naïve-treatment HIV-1 infected patients or newly diagnosed HIV patients, the third group, we could identified subtype CRF01_AE 75 patients (81.52%) and subtype B 17 patients (18.48%) by SSEIA. However, no significant difference of HIV-1 CRF01_AE subtype was mainly strain and followed by subtype B in HIV-1 infected patients between treatment-experienced patients and newly diagnosed HIV patients ($p > 0.05$).