HIV GENETIC VARIATION IN ANTIRETROVIRAL DRUG TREATED THAIS FROM 2009-2011

NATTAPOL NARONG

A THESIS SUBMITTED IN PARTIAL FULLFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY) FACULTY OF GRADUATE STUDIES MAHIDOL UNIVERSITY 2017

Thesis
entitled

HIV GENETIC VARIATION INANTIRETROVIRAL DRUG TREATED THAIS FROM 2009-2011

Nattapol Narong

Mr. Nattapol Narong
Candidate

Prof. Ruengpung Sutthent,
M.D., Ph.D. (Microbiology and Immunology)
Major advisor

Wannee Kantakamalakul

Prof. Wannee Kantakamalakul,
Ph.D. (Microbiology and Immunology)
Co-advisor

NAVIN HORTHONGKHAM

Asst. Prof. Navin Horthongkham,
Ph.D. (Microbiology)
Co-advisor

Prof. Patcharee Lertrit,
M.D., Ph.D. (Biochemistry)
Dean
Faculty of Graduate Studies
Mahidol University

Iyarit Thaipisuttikul

Asst. Prof. Iyarit Thaipisuttikul,
M.D., Ph.D. (Genetics)
Program Director
Master of Science Program in Microbiology
Faculty of Medicine Siriraj Hospital
Mahidol University

Thesis
entitled

HIV GENETIC VARIATION INANTIRETROVIRAL DRUG TREATED THAIS FROM 2009-2011

was submitted to the Faculty of Graduate Studies, Mahidol university
for the degree of Master of Science (Microbiology)
on
Mar29, 2017

Nattapol Narong
Mr. Nattapol Narong
Candidate

Palanee Amm
Asst. Prof. Palanee Ammaranond,
Ph.D. (Immunology)
Chair

R. Sut
Prof. Ruengpung Sutthent,
M.D., Ph.D. (Microbiology and
Immunology)
Member

NAVIN HORTHONGKHAM
Assist. Prof. Dr.Navin Horthongkham,
Ph.D. (Microbiology)
Member

Wannee Kantakamalakul
Prof. Wannee Kantakamalakul ,
Ph.D. (Microbiology and Immunology)
Member

Prof. Patcharee Lertrit,
M.D., Ph.D. (Biochemistry)
Dean
Faculty of Graduate Studies
Mahidol University

Prof. Prasit Watanapa,
M.D., Ph.D., FRCS, FACS
Dean
Faculty of Medicine Siriraj Hospital
Mahidol University

ACKNOWLEDGEMENTS

This success of this thesis can be attributed to the extensive support and assistance from people who became involved until this thesis to its completion. First and foremost, I would like to express thanks to my thesis advisor, Prof. Dr. Ruengpung Sutthent from Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University for her attention, excellent instruction, advice and valuable guidance throughout this thesis. Thank you for pushing me to succeed in my research. My special appreciation is deeply expressed to my co-advisor, Asst. Prof. Dr. Navin Horthongkham from Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University for his suggestion, encouragement and valuable advice. His guidance helped me in all the time of research and writing of this thesis.

Beside my advisor, I would like to special thank to chairperson of thesis defense, Assist. Prof. Dr. Palanee Ammaranond, from Faculty of Allied Health Science, Chulalongkorn University for her suggestions and constructive comments in this thesis. I also would like to thank the rest of my committee Prof. Dr. Wannee Kantakamalakul from Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University for their encouragement and insightful comments in this thesis.

My sincere thanks are due to staff of molecular laboratory at Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University for their help and support for this thesis and also grateful to my friends for their assistance and support during my studies in master degree program.

In particular, I would like to special thank to HIV/AIDS patients for their precious specimens for my thesis. Without their participation, I would have never been able to provide crucial information of HIV/AIDS epidemiology in Thailand which useful for planning more effective HIV prevention, treatment and care for HIV/AIDS patients.

Finally, my deepest gratitude is especially expressed to my family who provide the greatest love, willpower and continuous support throughout my life.

Nattapol Narong

HIV GENETIC VARIATION IN ANTIRETROVIRAL DRUG TREATED THAIS FROM 2009-2011

NATTAPOL NARONG 5436075 SIMI/M

M.Sc. (MICROBIOLOGY)

THESIS ADVISORY COMMITTEE: RUENGPUNG SUTTHENT, M.D., Ph.D., WANNEE KANTAKAMALAKUL, Ph.D., NAVIN HORTHONGKHAM, PhD.

ABSTRACT

AIDS is caused by human immunodeficiency virus (HIV) which can be divided into two major types, HIV type 1 (HIV-1) and HIV type 2 (HIV-2). The most common HIV in the world and also in Thailand is HIV-1. HIV type 1 can be divided into four distinct and highly divergent groups based primarily on the analysis of genetic sequences coding for the envelope (*env*) and other structural (*gag*, *pol*) proteins, including M , O , N and P. The variations of envelope proteins of HIV-1 group M and HIV-1 group O are 30%-50%. HIV-1 group M is the most widespread which can be recognized into several subtypes (A, B, C, D, F, G, H, J, and K). Within subtype A and F have been further divided into A1, A2 and F1, F2, respectively. The HIV-1 subtype B and CRF01_AE are the major subtypes circulating in Thailand.

This study aimed to analyze the genetic sequences of HIV from 675 collected clinical samples for detecting drug resistance genotype of HIV using nucleotide sequencing. Thirty-five of the 675 random samples of the collected samples from 2009 (n=111), 2010 (n=250) and 2011 (n=314) were selected using near full length genomic sequencing analysis. Moreover the researcher studied the prevalence of drug resistance mutation in all samples. The results revealed that phylogenetic tree of 32 out of 35 samples was shown in subtype CRF01_AE and the 3 remaining samples were clustered with CRF34_01B and CRF52_01B. In HIV drug resistance mutation prevalence study, the collected samples from 2009 showed the most drug resistance gene variation of NRTIs group in M184V/MV/IMV/IM/I (65.5%), NNRTIs group in Y181C/CY/G/FY/I/V/HY/L (37.8%) and PIs group in L63P (21.8%). The collected samples from 2010 revealed the most drug resistance genotyping variation of NRTIs group in M184V/MV/IMV/IM/I (49.31%), NNRTI group in Y181C/CY/G/FY/I/V/HY/L (34.31%), PIs group in L63P (19 %), while collected samples from 2011 showed the most drug resistance genotyping variation of NRTIs group in M184V/MV/IMV/IM/I (58.32%), NNRTI group in Y181C/CY/G/FY/I/V/HY/L (33.63%) and PIs group in M46I/L/IM/LM (2.63 %)

This study found that major subtype of HIV-1 circulated in Thailand CRF01_AE, which corresponds to the HIV epidemiology of Thailand and with a high percentages (8.6%) of CRF01/B reported here from samples collected from Southern, Northern and Northeastern provinces of Thailand. These samples might be used as preliminary data to urge for intensive molecular epidemiological study of HIV genotype spread in Thailand.

KEY WORDS: HUMAN IMMUNODEFICIENCY VIRUS/
NUCLEOTIDE SEQUENCING/ PHYLOGENETIC TREE/ CRF01/B
RECOMBINATION

105 pages

การเปลี่ยนแปลงสารพันธุกรรมของเชื้อเอชไอวี(HIV)ในผู้ป่วยคนไทยที่ได้รับยาต้านเชื้อเรโทรไวรัสระหว่าง ค.ศ. 2009-2011

HIV GENETIC VARIATION IN ANTIRETROVIRAL DRUG TREATED THAIS FROM 2009-2011

ณัฐพล ฉรงค์ 5436075 SIMI/M

วท.ม. (จุลชีววิทยา)

คณะกรรมการที่ปรึกษาวิทยานิพนธ์: รวงผึ้ง สุทเธนทร์, M.D., Ph.D., วรรณี กัณฐกมาลากุล, Ph.D., นาวิน ห่อทองคำ, Ph.D.

บทคัดย่อ

โรคเอดส์เกิดจากเชื้อไวรัสเอชไอวี โดยที่เชื้อเอชไอวีสามารถแบ่งออกได้เป็น2ชนิดคือเชื้อไวรัสเอชไอวีไทป์1และเชื้อไวรัสเอชไอวีไทป์2 ในประเทศไทยเชื้อเอชไอวีที่ระบาดอยู่นั้นจะเป็นชนิดไทป์1 เชื้อเอชไอวีไทป์ 1 สามารถแบ่งเป็น 4 กลุ่มคือกลุ่มM,N,OและPโดยใช้การวิเคราะห์จากสารพันธุกรรม โดยที่เชื้อเอชไอวีกลุ่ม M พบว่ามีการระบาดมากที่สุด โดยที่กลุ่มMสามารถแบ่งได้อีก 9 ซับไทป์คือ A, B, C, D, F, G, H, J, และ K ในซับไทป์ A และ F ยังแบ่งเป็น A1, A2 และ F1, F2 ในประเทศไทยซับไทป์ที่ระบาดมากคือ B และCRF01_AE

การศึกษาในครั้งนี้ได้ศึกษาโดยใช้การวิเคราะห์ลำดับเบสของเชื้อเอชไอวี โดยใช้ตัวอย่างจากผู้ติดเชื้อเอชไอวีที่ทำการส่งตรวจหายีนดื้อยาของเชื้อเอชไอวีในภาควิชาจุลชีววิทยาคณะแพทยศาสตร์

โรงพยาบาลศิริราช การศึกษาครั้งนี้ได้ทำการสุ่มเลือกตัวอย่างทั้งหมด 35 ตัวอย่างจาก 675 ตัวอย่าง(ค.ศ. 2009จำนวน 111ตัวอย่าง, ค.ศ. 2010 จำนวน250 ตัวอย่างและค.ศ. 2011 จำนวน 314 ตัวอย่าง) นำไปทำการวิเคราะห์ทางลำดับเบส จากการวิเคราะห์พบว่าตัวอย่างจำนวน 32 ตัวอย่างเป็นซับไทป์ CRF01_AE และมีตัวอย่าง 3 ตัวอย่างเกิดการผสมระหว่างซับไทป์ CRFO1_AE และ B โดยอยู่ในกลุ่ม CRF34_01B และ CRF52_01B ในส่วนการศึกษาความชุกของการดื้อยาพบว่าในปีค.ศ.2009 กลุ่มยาNRTIs, NNRTI, PIs ตำแหน่งยีนที่พบว่าเกิดการเปลี่ยนแปลงมากคือตำแหน่ง M184V/MV/IMV/IM/I (65.5%), Y181C/CY/G/FY/I/V/HY/L (37.8%)และ L63P (21.8%) ตามลำดับ ปีค.ศ.2010 กลุ่มยา NRTIs, NNRTI, PIs ตำแหน่งยีนที่พบว่าเกิดการเปลี่ยนแปลงมากคือตำแหน่ง M184V/MV/IMV/IM/I (49.31%), Y181C/CY/G/FY/I/V/HY/L (34.31%)และ L63P (19%) ตามลำดับและปีค.ศ.2011 กลุ่มยา NRTIs, NNRTI, PIs ตำแหน่งยีนที่พบว่าเกิดการเปลี่ยนแปลงมากคือตำแหน่ง M184V/MV/IMV/IM/I (58.32%), Y181C/CY/G/FY/I/V/HY/L (33.63%)และ M46I/L/IM/LM (2.63 %) ตามลำดับ

จากการศึกษาพบว่าเชื้อเอชไอวี 1 ที่ทำการศึกษาเป็นซับไทป์ CRFO1_AEและ B ซึ่งสอดคล้องกับการระบาดของเชื้อเอชไอวีในประเทศไทยและการที่พบอัตราส่วนที่สูงของซับไทป์ CRF01/B(8.6%) จากตัวอย่างที่ได้มาจากภาคใต้ ภาคตะวันออกเฉียงและภาคเหนือของประเทศไทยอาจเป็นข้อมูลเบื้องต้นในการศึกษาระบาดวิทยาของเชื้อเอชไอวีในประเทศไทยอย่างละเอียดถี่ถ้วน

105 หน้า

CONTENTS

CONTENTS (cont.)

LIST OF TABLES

LIST OF TABLES (cont)

LIST OF FIGURES

LIST OF ABBREVIATION

AIDS	Acquired immune deficiency syndrome
HIV	Human immunodeficiency form
CRF	Circulating recombinant from
URF	Unique recombinant form
M	Major
N	New
O	Outlier
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
Pro	Protease
LTR	Long terminal repeat
NJ	Neighbor joining
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
PIs	Protease inhibitors
HAART	Highly active antiretroviral therapy
cDNA	Complimentary deoxyribonucleic acid

CHAPTER I
INTRODUCTION

Since the first reported case of Acquired Immune Deficiency Syndrome (AIDS) in 1983, there were 25 million people have been killed and 36.1 million are living with HIV infection. In 2009, World Health Organization (WHO) reported a global estimated number of HIV infection was about 33.4 million people and the number of newly adults infected with HIV was approximately 2.6 million per year (3). In Asia, 4.9 million people were infected with HIV and there were 360,000 people newly infected with HIV each year. In Thailand, there are 530,000 people (1.3%) of adult infected with HIV during 2009 (3). Recently, the Bureau of epidemiology, Department of Disease Control Ministry of Public Health, Thailand, reports the total number of people who living with HIV infection, since 1984 to November 15th, 2011, were 376,690 people. Among these, 83.99% were attributed to unprotected sexual intercourse, 4.41% were injecting drug use, 3.59% were mother-to-child transmission, and 0.03% was receiving infected blood (4).

Human immunodeficiency virus can divide into two types, HIV-1 and HIV-2; both types are causing clinically indistinguishable AIDS. HIV-1 viruses are divided into 4 distinct groups based on *env* gene, designated group M, N, O and P (5). The HIV-1 group M virus that dominates the AIDS pandemic can be further subdivided into 9 subtypes. These aresubtypes A, B, C, D, F, G, H, J, and K. There are also circulating recombinant forms or CRFs, a new hybrid virus derived from recombination between viruses of different subtypes, of which at least 48 have been identified. Genetic distance of different subtypes are ranging from 20-35% and 7-20% of the same subtypes (6). The HIV-1 subtypes and CRFs pandemic are uncertain within regions, with typically related in some countries. Subtype C is the dominant form causing approximately 48% of HIV-infection worldwide and commonly found in India, China, and South Africa. Subtype B is generally found in North and South America, Europe and Australia causing 11% of HIV infection globally. Subtype A

(12%) and CRF02_AG (8%) have been found in West and South Africa. Subtype CRF01_AE (5%) is common in Southeast Asia including Thailand. Subtype G (5%) is primarily found in West and Central Africa. Subtype D (2%) is limited to East Africa whereas subtype F is circulated worldwide. Subtype H, J, and K have only been found least than 1% in Central, South, and Western Africa (7). At the initial phase of HIV epidemic in Thailand, subtype B was predominantly found in injection drug user. However, the circulated HIV-1 strains were inconsistence; CRF01_AE became the strain that commonly found in newly infected patient both from injecting drug use and sexual transmission. The study of circulating strains of HIV-1 in all geographic region of Thailand; rely on *env* variation, during 1995-2004 showed the 97.3% prevalence of subtype CRF01_AEand 2.7% of subtype B. Recently, there was investigation about HIV-1 epidemic in northern Thailand based on genotyping of *pol* gene reveled that subtype CRF01_AE has been found 94.8%, subtype B 2.9%, and subtype C 0.5%. In addition, the study of HIV-1 in Bangkok and the nearly provinces according to *pol* gene exposed the emergence of 86.9% for subtype CRF01_AE, 8.6% for subtype B, and 4.5% were the other recombinant strains.

The critical factors that induced the spreading of HIV worldwide compose of the diversity of the organisms themselves and also the rapid evolution from spontaneous mutation. HIV diversity and evolutionary change are induced by 3 main mechanisms; 1) the infection of various HIV subtypes in the population (8-10), 2) the defect of reverse transcriptase enzyme also the efficiently recombination event (11-14), and 3) the highly reproductive rate of HIV (15-16). Because of the variation, HIV can evade the immune response, causing the resistant strain with sequential treatment failure (17), and also effect disease progression (18-19).

HIV genetic variability can occur in several genes such as *env, gag, pol*, or LTR. The most variable region is *env* gene resulting in different capability to target and attach to specific cell types and also associate with the HIV subtype classification. Several studies revealed that the modification in V3 loop, a part of the virion's envelope glycoprotein, effect the infiltration to the target cell membrane (20). In addition, different subtype influenced different disease progression and severity; subtype D induced a faster disease progression than subtype A, for instance (21, 22). Although genetic variation of *pol* gene occurs 2-3 times less than *env* gene, it play an

important role in drug resistant. Isolates of subtype D exhibit a lower susceptibility to all antiviral drugs than subtype A, B, C , and CRF01_AE (23). This drug resistant mutation is transmittable and associate with HIV disease progression also (24). Since HIV genes are extremely variable, it is an enormous obstacle to design an effective vaccine against HIV. The appropriate antigen for vaccine development should be considered about the epidemic strain in that region and also HLA subtype in the population.

Because the elaboration of HIV diversity in Thailand is important information that may describe the evolution and variation of HIV, this study aim to investigate and differentiate HIV genome variation from patients admitted to Siriraj Hospital during January 2009 to December 2011. The specimens were delivered to the Department of Microbiology, Siriraj Hostpital for drug susceptibility testing by *env*, *gag*, and *pol* sequencing. This knowledge is a guideline for the appropriate signature and epitope for CRF01_AE vaccine improvement and reveals antiviral resistant situation.

CHAPTER II
OBJECTIVES

1. To investigate molecular epidemiology of human immunodeficiency virus (HIV) among HIV infected samples sent to Siriraj Hospital from January 2009 to December 2011.

2. To determine prevalence of drug resistance mutations of HIV-1 reverse-transcriptase (NRTI, NNRTI) and protease (PI)) genes.

3. To study the variation of *env, gag* and *pol* genes in HIV-infected individual who receives antiretroviral therapy.

CHAPTER III
LITERATURE REVIEW

3.1 Discovery of HIV

In 1981, several case emergence of Kaposi's Sarcoma and Pneumocystis among gay men in New York and California. When the Centers for Disease Control reported the new outbreak they called it "GRID" (gay-related immune deficiency) (25) stigmatizing the gay community as carriers of this deadly disease. However, cases started to be seen in heterosexuals, drug addicts, and people who received blood transfusions, proving the syndrome knew no boundaries. In 1982, The Centers for Disease Control (CDC) establishes the term acquired immunodeficiency syndrome (AIDS) and identifies four "risk factors": male homosexuality, intravenous drug use, Haitian origin, and hemophilia A. From the report review that in 1983, Aids is major outbreak in both men and women in Central Africa and the Pasteur Institute in France, Drs. Francoise Barr-Sinoussi, Luc Montagnier, et al., isolate a new retrovirus from a French patient with AIDS symptoms. They recovered a reverse-transcriptase containing virus from the lympnode of a man persistent lymphadenopathy syndrome (LAS). At the time, some physicians suspected that this syndrome was associated with AIDS. Gallo and co-workers isolate human T-cell like leukemia virus (HTLV) (26) from individuals with AIDS. Then, Montagnier and co-workers can show result indicated that virus can replicate in CD4+ cells and killed CD4+ cells. In 1984, Levy and co-workers report can identification of retroviruses they named the AIDS-associated retroviruses (ARV). ARV recovers in different risk groups, other symptomatic and some healthy people. After that, LAV, HTLV and ARV were recognized as members of the same group of Retroviruses in Lentivirinae family. Soon after the discovery of HIV-1, a separate virus type, HIV-2, was identified in western Africa (27).

3.2 Structure and genomes HIV-1

Human immunodeficiency viruses (HIV) belong to the genus Lentivirus part of the Retroviridae family. It is an enveloped virus with particles that are 80-130 nm in diameter. Within capsid HIV have two copies packages of positive-sense RNA strands in its genomes. HIV RNA genomes use reverse transcriptase molecules for convert the RNA genome to DNA upon infection of the host. The capsid enclosed the genome and gives the virus its helical or icosahedral. Core of HIV is cone shape that belong to genus Lentivirus. The outer shell is viral envelope (ENV), consist of a lipid bilayer that is acquired as the virus buds from the cell surface. Viral ENV proteins are encode by the viral genome and often appear on the envelope as projection known a spike. The viral envelope is a complex protein that is gp120/gp41 with an important role in viral entry. Inside the viral ENV is a protein called p17 (matrix, MA) and within viral core called p24 (core antigen, capsid, CA) (Figure 3.1) (28).

The HIV-1 viral genome is monomer 7 to 13 kilobases (kb) in size, with open reading frames coding for several viral proteins (Table 3.1). Viral genomic RNA is present as a homodimer of two identical sequence. HIV has several major genes coding for structural proteins. Both ends of the provirus are flanked by a repeated sequence known as the long terminal repeats (LTRs). HIV genome is consist ten genes. Three genes providing genetic information for HIV structural components are terms *gag*, *pol* and *env*. The *gag* and *env* genes encode the capsid protein (Gag) and external glycoprotein (ENV) and *pol* gene encode the viral enzyme necessary for replication. Furthermore, HIV contain seven accessory gene such as Virion Infectivity Factor (*vif*), Viral Protein U (*vpu*), Viral Protein R (*vpr*), Trans-Activator of Transcription (*tat*), Regulator of Virion protein expression (*rev*), Negative Regulatory Factor (*nef*) and *tev* gene (It is a fusion of parts of the *tat*, *env*, and *rev* genes) (Figure 3.2).

The primary transcript of HIV is a full–length viral mRNA that is translated into the Gag and Pol protein. The Gag–Pol precursor of 160 kDa is processed by the viral protease. By proteolytic cleavage, the Gag precursor pr55 gives rise to the smaller proteins (MA, p17; CA, p24; proline-rich, p6; NC, p9). The Pol precursor protein is cleaved into products consisting of the viral enzymes; protease (PR, p10), reverse transcriptase/RNase H (RT, p66 p51), and integrase (IN, p32). The

Env precursor (gp 160) is processed by cellular protease into the surface glycoprotein (SU, gp120) and transmembrane glycoprotein (TM, gp41) (Figure 3.3).

Gene products of other spliced mRNA make up a variety of viral regulartory and accessory proteins; Tat (p24), Tev (p26), Rev (p19), Nef (p27), Vif (p23), Vpr (p15), and Vpu (p16) that can effect HIV replication in various cell types.

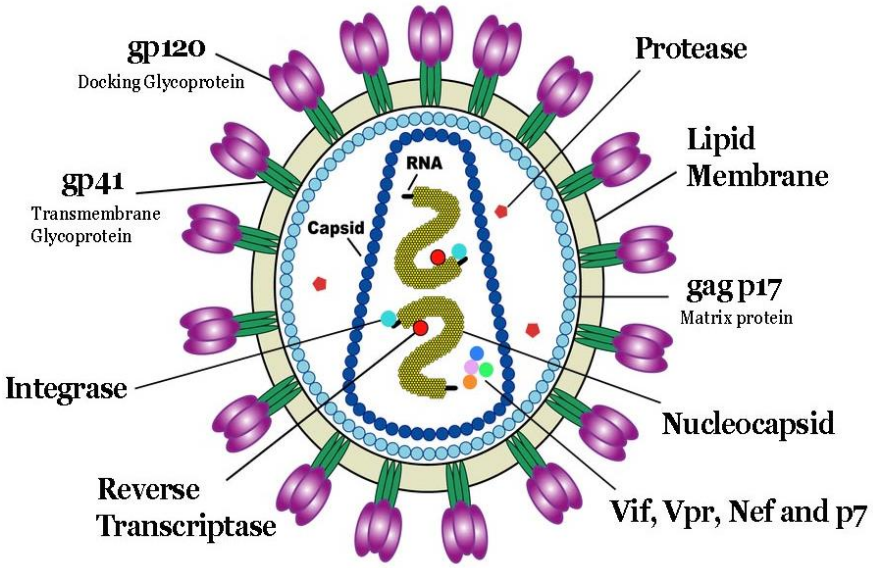


Figure 3.1: Schematic representation of HIV-1 virion (28)

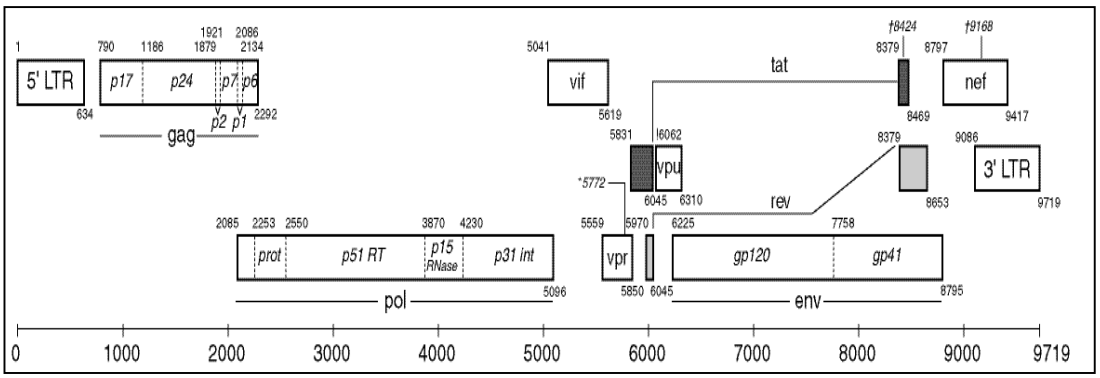


Figure 3.2: Genomic structure of HIV-1 (29)

Table 3.1 Protein function from HIV-1genes

Name	Size	Function	Localization
Gag MA	p17	Membrane anchoring, Env interaction, nuclear transport of viral core(myristylated protein)	Virion
Gag CA	p24	Core capsid	Virion
Gag NC	p7	Nucleaocapsid, binds RNA	Virion
	p6	Binds Vpr	
Protease	p15	Gag-Pol cleavage and maturation	Virion
Reverse transcriptase, RNase H	p66 p55 (heterodimer)	Reverse transcription, RNase H activity	Virion
Integrase		DNA provirus integration	Virion
Env	gp120/gp41	External viral glycoproteins bind to CD4 receptor	Plasma membrane, virion envelope
Tat	p16/p14	Viral transcriptional transactivator	Primarily in nucleolus /nucleus

Table 3.1 Protein function from HIV-1genes (cont.)

Name	Size	Function	Localization
Rev	p19	RNA transport, stability and factor (phosphoprotein)	Primarily in nucleolus/ nucleus use shuttling between nucleolus and cytoplasm
Vif	p23	Promotes virion maturation and infectivity	Cytoplasm (cytosol membranes), virion
Vpr	p10-15	Promotes nuclear localization of preintegration complex, inhibits cell division, arrests infected cells at G2 /M	Virion, nucleus
Vpu	p16	Promotes extracellular release of viral particles, degrades CD4 in the endoplasmic reticulum (phosphoprotein)	Integral membrane protein
Nef	p27/p25	CD4 downregulation (myristylated protein)	Plasma membrane, cytoplasm
Vpx	p12-p16	Virion protein, vpr homolologue	Virion
Tev	p28	Tripartite Tat-Env-Rev protein (also named Tnv)	Primarily in nucleolus/nucleus

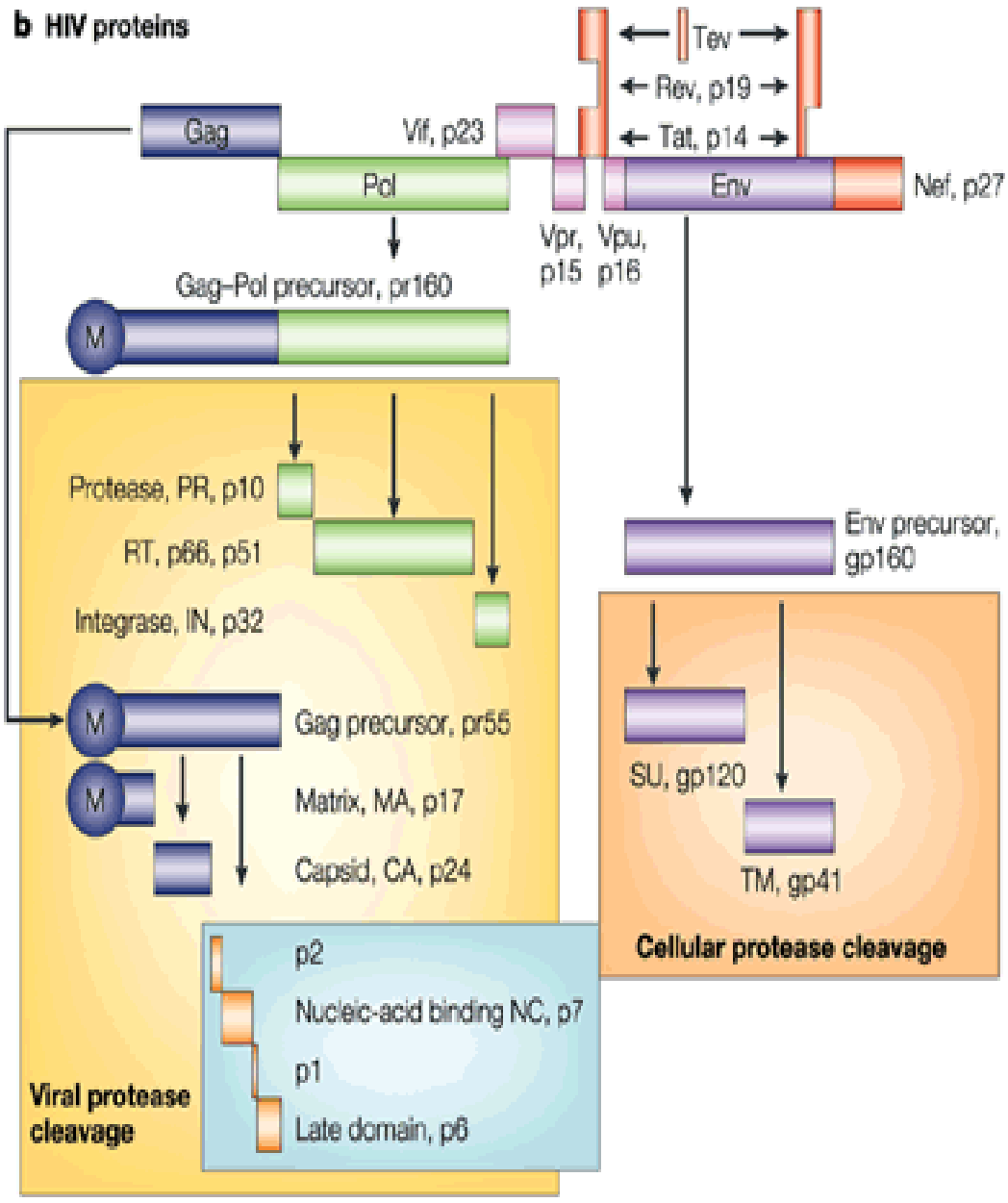


Figure 3.3: Processing of viral proteins (30)

3.3 The HIV-1 Replication Cycles (Figure 3.4)

Unlike others RNA virus, HIV showed a unique life cycle that comprises the series of events referred as the early and the late phase. The early phase start from the initial attachment of the virus to the host cell surface, the viral envelope fuses with the cell membrane through the integration of the viral genome within the host genome. Then the steps from the expression of the viral genome until the production of new virus particles that bud off from the host cell are called the late phase.

The cells mainly targeted by HIV are macrophages, CD4+ T cells and dendritic cell. The virus particle enters the target cells by the binding of viral enveloped gp120 to CD4 receptor (31) on the cellular membrane leading to conformational change of gp120 exposing the chemokine binding domains which allows for secondary interaction to a co-receptors, either CCR5 or CXCR4 depending on the strains of virus. The gp41, another glycoprotein on viral envelope spike, are then inserted into the host membrane and undergoes significant conformational changes that induce the fusion of the viral and cellular membrane.

After viral fusion, viral RNA and various viral enzymes are injected into the cell. Similar to other retroviruses, HIV contains reverse transcriptase enzyme that converts the single-stranded HIV RNA to double-stranded DNA. The newly formed HIV DNA enters the host cell's nucleus, where the enzyme integrase facilitates the integration of this viral DNA into the host cell’s DNA. The integrated HIV DNA is called provirus. Provirus is replicated along with the chromosome when the cell divides to create copies of the HIV genomic material, as well as messenger RNA which is used to synthesis of HIV proteins. The provirus may remain inactive for several years, producing few or no new copies of HIV and provides the latency that enables the virus to evade host responses so effectively. Nevertheless, the HIV proviral genome can be activated by the certain cellular transcription factors such as NF-κB which is upregulated when T-cells become activated. This incident cause by the cells that almost to be killed by HIV is concurrently fighting infection.

After the HIV genome is activated, the integrated provirus is transcribed into mRNA and be modified for the production of regulatory and structural proteins. Nonetheless, viral genome has to be transported out of the nucleus without any

modification as the full-length RNA. The processing HIV proteins come together with copies of HIV's RNA genetic material, a new virus particle is assembled.

The newly assembled virus particles bud from the surface of the cell and taking part of the host cell membrane as the new viral envelope. The new copies of HIV then move to infect other cells for the next replication cycle (32, 33).

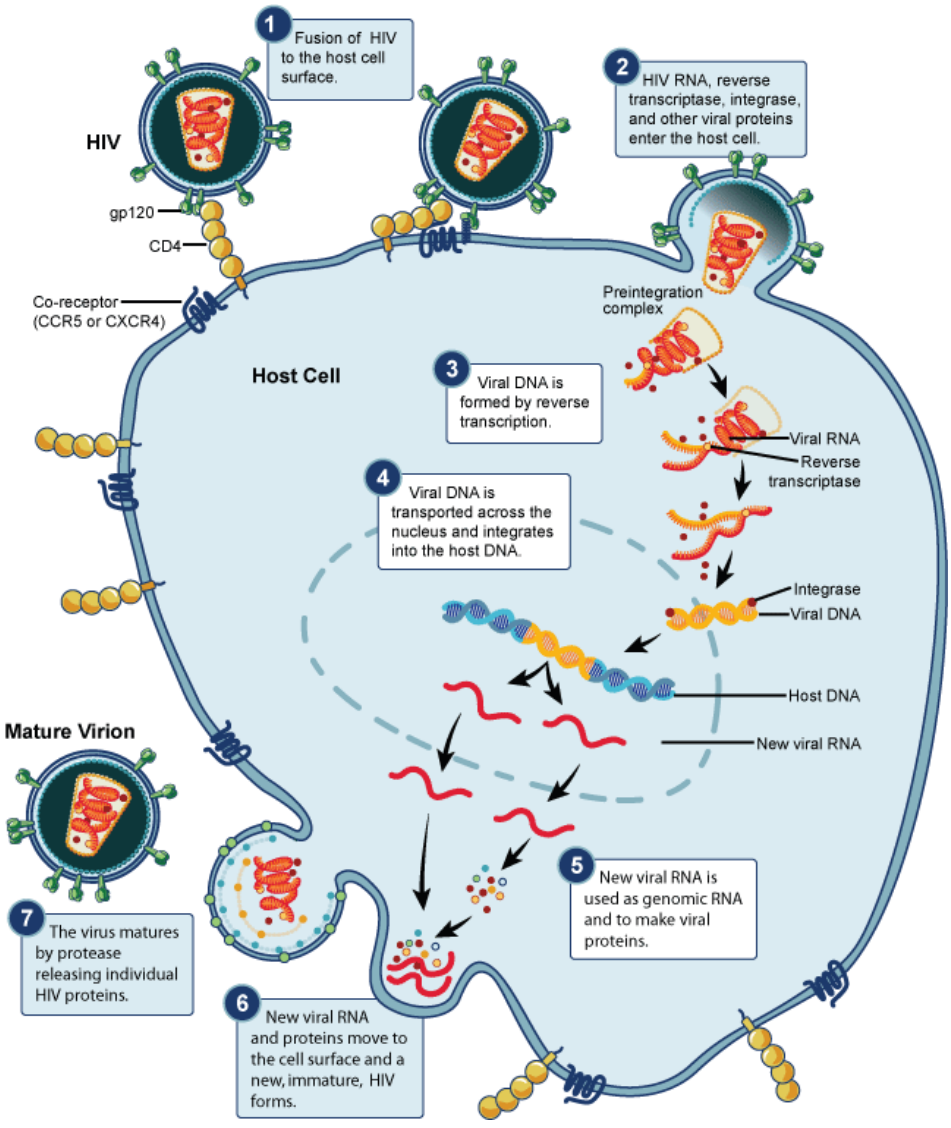


Figure 3.4: General features of the HIV-1 replication cycle (34)

3.4 HIV-1 classification

Four types of categories should be used to refer to the major HIV-1 lineages: groups, subtypes, sub-subtypes and CRFs.

Groups will continue to refer to the very distinctive HIV-1 lineages M, N and O Group M includes the viruses that dominate the global epidemic and the sub-division of this group is the focus of this proposal. The groups were originally named M for main, O for outlier, and N for Non-M-Non-O (Figure 3.5).

Subtypes will continue to refer to the major clades within group M.

Sub-subtype will be used to refer to a distinctive lineage that is very closely related to a particular subtype lineage, and is not genetically distant enough to justify calling a new subtype.

Circulating Recombinant Form (CRF) describes a recombinant lineage, that plays an important role in the HIV-1 pandemic. The CRF members must share an identical mosaic structure, *i.e.*, they are descended from the same recombination event(s) and breakpoint where recombination occurred should be identified.

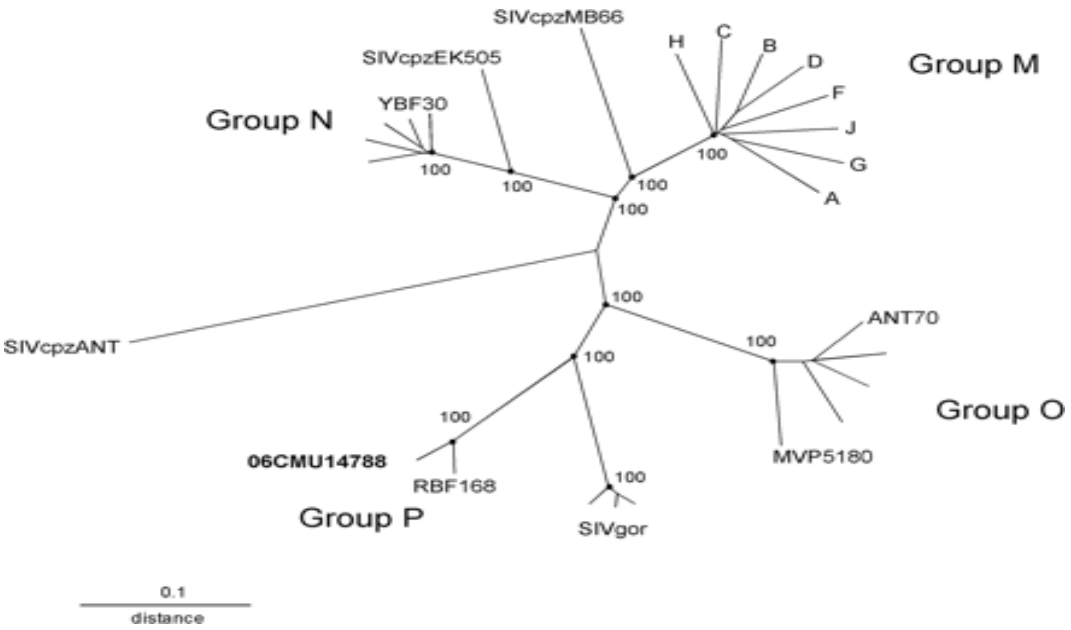


Figure 3.5: Phylogenetic relationships between group M, N, O and P (35)

HIV-1 can be divided into 4 distinct groups, M (main), O (outlier), N (non-M, non-O) and P (Figure 5). Groups N and O are rare, and largely restricted to Cameroon

and surrounding countries. The vast majority (perhaps 98%) of HIV infections worldwide are caused by HIV-1 group M (36). The HIV-1 M group subtypes are phylogenetically associated groups or clades of HIV-1 sequences and are labeled A1, A2, B, C, D, F1, F2, G, H, J and K. Two of the CRF (CRF01_AE and CRF04_cpx) were classified initially as subtypes (‘‘E’’ and ‘‘I’’, respectively). Within A and F subtypes, separate subclusters are distinguished, designated sub-subtypes A1 , and A2, and F1 and F2, respectively, each pair of sub-subtypes being more closely related to each other than with other subtypes (37). HIV-1 subtypes, also called clades, are phylogenetically linked strains of HIV-1 that are approximately the same genetic distance from one another; in some cases, subtypes are also linked geographically or epidemiologically. Genetic variation within a subtype can be 15 to 20%, whereas variation between subtypes is usually 25 to 35%. Over the past decade, advances in full-genome sequencing of HIV have led to the identification of circulating and unique recombinant forms (CRFs and URFs, respectively) (38). The identification of these subtypes and CRFs is important in epidemiological tracking and in the understanding of the ever-changing epidemic. Now, four subtypes and two CRFs dominate the global epidemic: subtypes A through D, CRF01_ AE and CRF02_ AG (28). HIV group N is a very distinctive form of the virus that has only been identified in a few individuals in Cameroon. Group N is sometime referred to as Not-M, Not-O, also sometimes as the "new" group, and is also thought to have originated from a chimpanzee transmission. Subtypes within the HIV-1 N group are not yet clearly defined. Very few isolates have been identified and sequenced. HIV-1 Group O, sometimes referred to as the "outlier" group, like group M contains very diverse viruses, but is still relatively rarely found. It is thought to have originated in a transmission to humans from wild gorilla. Incidence of HIV-1 group P is quite low, which was reported to be found in a Cameroonian woman living in France and patient in Cameroonian hospital at Cameroon. The group P viral sequence, RBF168, forms a distinct HIV-1 lineage that includes SIV sequences from western gorillas (SIVgor; *Gorillagorilla gorilla*), suggesting that group P originated from gorillas (29).

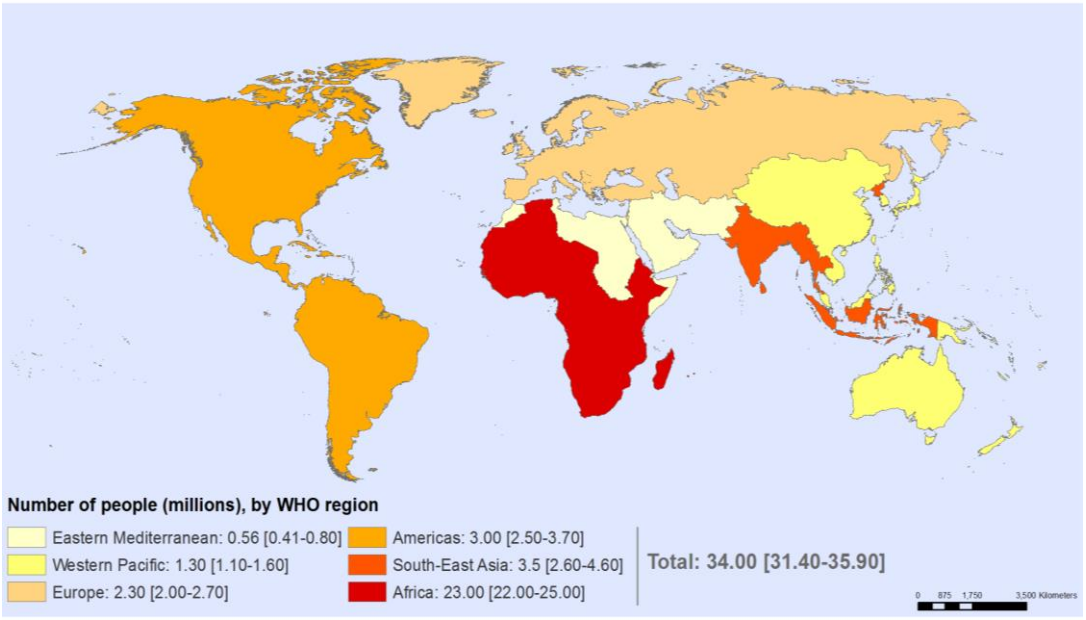


Figure 3.6: Adults and children estimated to be living with HIV, 2011 (39)

HIV-1 infection cause one of the world's most devastating impacts. Since the first cases reported in 1981, the cumulative total of HIV infections and deaths exceeds 60 million and 30 million, respectively. The global estimated number of HIV infected in 2011 was approximately 34.0 million with 1.7 million of annual deaths (Figure 3.6) (40).

HIV-1 group M strains responsible for the vast majority of HIV infections worldwide that include subtypes A, B, C, D, two CRF, CRF01_AE and CRF02_AG which dominate the global epidemic. Subtype C accounted for nearly half (48%) of all global infections concentrated in Southern and East Africa, and in India. Subtype A which are commonly found in East Africa and in former Soviet Republics caused 12% of infection while subtype B dominating epidemics in North American, the Caribbean, Latin America, Western and Central Europe and Australia account for 11% of global infection. Followed by, CRF02_AG (8%) that dominates west Africa with smaller number in Central Africa, the Middle East and North Africa, CRF01_AE (5%) the major strain throughout South and Southeast Asia, Eastern Asia and a small number in Central Africa. Subtype D (2%) is present mainly in Eastern Africa, with further infections in Central and West Africa.

Even the first AIDs cases were reported among men who have sex with men and in the early infection rates of homosexual population were nearly 100%, the cumulative cases was decreased to 42% in 2006 indicates drastically changed and the extent to entered the heterosexual population. In a heterosexual relationship which not related to injection drug use approximately 15% cumulative HIV/AIDS cases were reported from the epidemic's beginning up to December 2006 which showed a significant number of cases among older individuals. HIV also represent highly prevalent among injection drug user attributes in the range of 16 to 17% of the AIDs case each year with a peak rate of 27% in 2003.

The majority of HIV strains circulating in Southeast Asia are represented by CRF01_AE with an increasing number of recombinant forms with B and C subtypes. CRF01_AE dominates in Indonesia, Thailand, Cambodia, Laos, Myanmar, and Viet Nam. In Malaysia, so-circulation of CR01_AE and subtype B has resulted in the emergence of CRF33_01B in approximately 20% of its HIV-1 infected individuals, now described in Indonesia (49).At the initial phase of HIV epidemic in Thailand, subtype B was predominantly found in injection drug user (41, 42). However, the circulated HIV-1 strains were inconsistence; CRF01_AE became the strain that commonly found in newly infected patient both from injecting drug use and sexual transmission. The study of circulating strains of HIV-1 in all geographic region of Thailand; rely on *env* variation, during 1995-2004 showed the 97.3% prevalence of subtype CRF01_AE and 2.7% of subtype B (42). Recently, there was investigation about HIV-1 epidemic in Northern Thailand based on genotyping of *pol* gene revealed that subtype CRF01_AE has been found 94.8%, subtype B 2.9%, and subtype C 0.5% (43). In addition, the study of HIV-1 in Bangkok and the nearly provinces according to *pol* gene exposed the emergence of 86.9% for subtype CRF01_AE, 8.6% for subtype B, and 4.5% were the other recombinant strains (44). In 2011, approximately 376,690 cases were the total number of people who living with HIV infection, since 1984 to November 2011. Among these, 83.99% attributed to unprotected sexual intercourse, 4.41% were injecting drug use, 3.59% were mother-to-child transmission, and 0.03% was receiving infected blood. At the initial phase of HIV epidemic in Thailand, subtype B was predominantly found in injection drug user. However, the circulated

HIV-1 strains were inconsistence; CRF01_AE became the strain that commonly found in newly infected patient both from injecting drug use and sexual transmission.

3.5 Antiretroviral Drugs

The drugs (Table 3.2) used for treat HIV-type 1 infection belong to four distinct classes.

3.5.1 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)

The first class of antiretroviral drugs to be used in HIV disease were NRTIs (44). NRTIs stand for nucleoside RT inhibitors. Groups of drug is triphosphate form, compete with natural substrates and function as chain terminators. They can divided into two categories: the thymidine derivatives and non–thymidine. The two thymidine derivatives are zidovudine (ZDV) and stavudine (D4T). The second agent is generally selected from among lamivudine (3TC), didanosine (ddI) and zalcitabine (ddC). (45)

3.5.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

NNRTIs binding in a conformational change in the reverse transcriptase that distorts the positioning of the residues that bind DNA, inhibiting polymerization. Drug of NNRTIs are three licensed such as nevirapine (NVP), delavirdine (DLV) and efavirenz (EFV). There are similar activity.

3.5.3 Protease Inhibitors (PIs)

HIV-1 encodes a protease (PR) that is responsible for the posttranslational processing of the viral *gag* and *gag–pol* polyprotein gene for product the structural proteins and enzyme of the mature viral particle. Drug of PIs are effective because they can specifically inhibit HIV protease. Drugs can bind protease enzyme active site that are similar to the normal HIV polyprotein substrate.

3.5.4. Entry/Fusion Inhibitors

Drug of group designed molecule block the attachment of HIV gp120 to either the CD4 T cell receptor or the CCR5/CXCR4 co-receptors. Enfuvirtide (ENF) is the first in group. It action by blocking the conformational change in gp41 molecule that allow fusion of the virus's lipid bilayer and the cell membrane.

Thai national guideline for antiretroviral therapy in 2011 defined the application of ART regimens for three different HIV infected groups, pediatric infected, adults infected, and pregnant infected individuals.

The usage of antiretroviral therapy in pediatric patients should be started as soon as HIV diagnosis is confirmed in all symptomatic individuals whose ages are more than one year old. In case of minor symptomatic patients, initiation of ART should rely on age-specific CD4 thresholds (Table 3.3). The first-line ART regimen composes of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) (Table 3.4). Besides the most common NRTI combination, zidovudine (ZDV) and lamivudine (3TC), stavudine (d4T) and lamivudine (3TC) are also applicable. Since AZT has the less long-term toxicities, it becomes the favorable drug of choice compared to stavudine. The application of NNRTI is based on age groups, nevirapine (NVP) is preferred for children age < 3 years, and efavirenz (EFV) for older children. Since NVP is also used in prevention from mother to child transmission thus infants who previously exposed to NVP may harbor NVP resistance phenotype. Therefore, the suitable regimens should be two NRTIs and the protease inhibitor (PI) for infants, lopinavir (LPV/r). In case of treatment failure, as indicated by >2,000 copies/ml of the viral load from a genotypic analysis, the new treatment regimen should be apply (Figure 3.7)(70).

Table 3.2 Antiretroviral agents used in the treatment of HIV infection (46)

Drugs	Mechanisms of Action	Mechanisms of Resistance
Nucleoside analogues Zidovudine Stavudine Lamivudine Didanosine Zalcitabine Abacavir	Analogues of normal nucleosides Active as triphosphate derivatives Incorporated into nascent viral DNA Prematurely terminate HIV DNA synthesis	Thymidine analogue mutations promote ATP-mediated and pyrophosphate-mediated excision of the incorporated terminator M184V or Q151M complex mutations impair incorporation of nucleoside analogues
Nucleotide analogues Tenofovir	Same as nucleoside analogues	K65R impairs incorporation of tenofovir into DNA Thymidine analogue mutations often associated with cross-resistance to tenofovir
Nonnucleoside reverse-transcriptase inhibitors Nevirapine Efavirenz Delavirdine	Bind a hydrophobic pocket of HIV type 1 reverse transcriptase Block polymerization of viral DNA Inactive against HIV type 2	Mutations reduce affinity of the inhibitors for the enzyme Single mutations generally sufficient to induce high level of resistance
Protease inhibitors Saquinavir Ritonavir Indinavir Nelfinavir Amprenavir Lopinavir	Structure derived from natural peptidic substrates of the HIV type 1 protease Bind the active site of the protease	Mutations reduce affinity of the inhibitors for the enzyme High-level resistance requires accumulation of mutations
Fusion inhibitors Enfuvirtide	36-Amino-acid peptide derived from the HR2 domain of glycoprotein 41 Interferes with glycoprotein 41–dependent membrane fusion	Mutations affect HR1, a domain of glycoprotein 41 whose interaction with HR2 promotes membrane fusion

Table 3.3 Criteria for initiation of antiretroviral therapy among HIV-infected children

	Age <1 year	Age 1-5 years	Age > 5 years
Clinical staging criteria	All	CDC category B, C or WHO stage 3, 4	CDC category B, C or WHO stage 3, 4
Immunological criteria %CD4 or CD4 cell count	All	%CD4 <25	CD4 <350 cells/mm^3

Table 3.4 The recommended first line regimen in Thai children

	Age <3 years	Age >3 years
Preferred regimens	AZT+3TC+NVP	AZT+3TC+EFV
Preferred regimens for adolescents (weight >40 kg or Tanner stage IV)	-	TDF+3TC+EFV
Alternative regimens	d4T+3TC+NVP	AZT+3TC+NVP d4T+3TC+EFV d4T+3TC+NVP

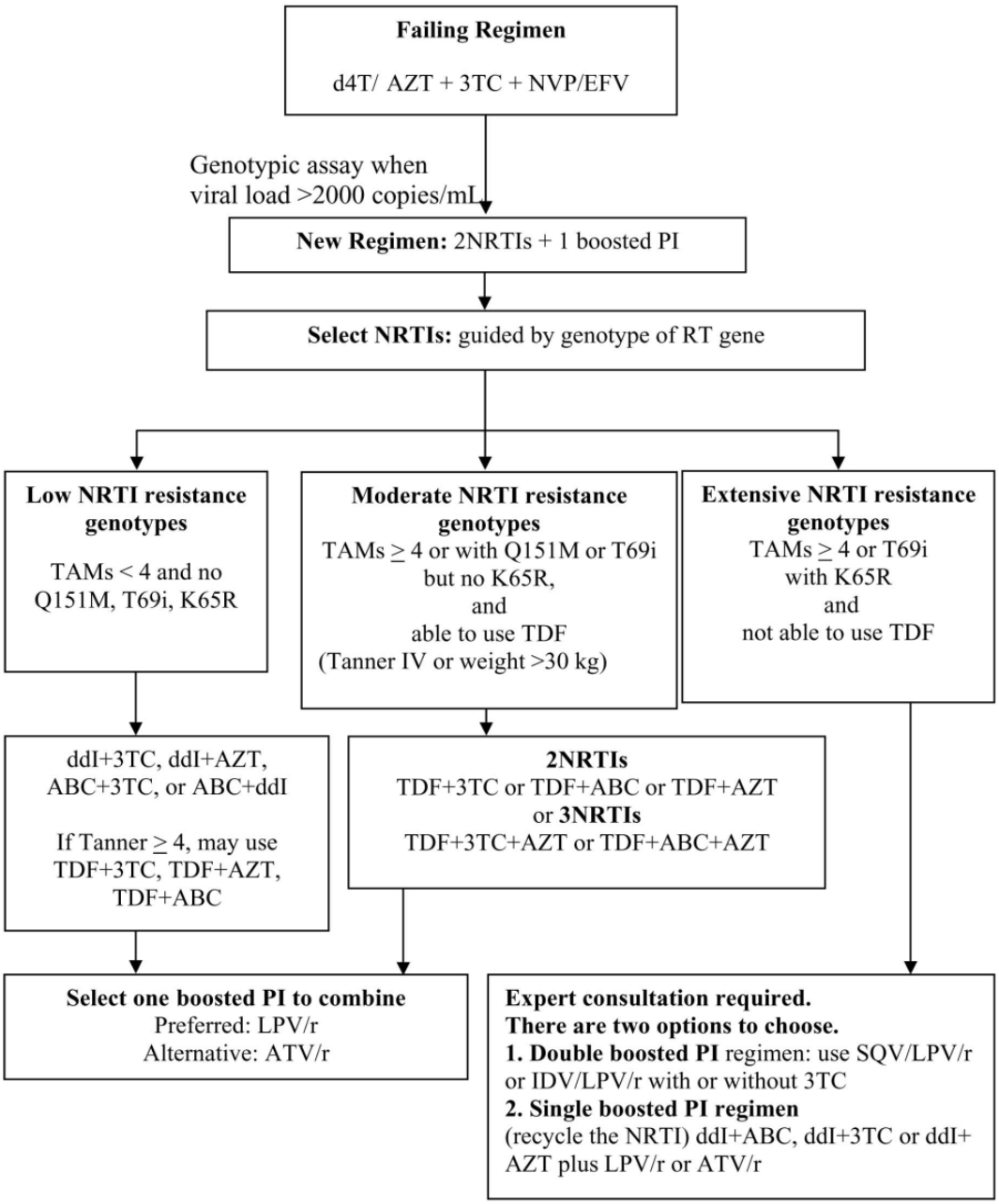


Figure 3.7: A new or salvage regimen in children failing 2NRTI+NNRTI regimens

Antiretroviral therapy for treatment HIV-infected adults and adolescents is relied on patient's symptoms and CD4+ T-cell counts (Table 3.5). The recommended first-line treatment is non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens, which are NVP, an aravirenz (EFV), and ritonavir-boosted lopinavir (LPV/r). EFV-based regimen is not recommended in the first trimester pregnancy women or in sexually active women who are not using contraception. LPV/r is usually applied as an alternative regimen in patients who cannot tolerate NVP and/or EFV treatment. If the treatment cannot achieve the ultimate aim with a VL level of <50 copies/ml, the next ART regimen will be planned (Table 3.6).

Table 3.5 Indications for initiation of antiretroviral therapy (ART)

Clinical Presentation	CD4+ T-cell counts (cells/mm^3)	Recommendations
AIDS-defining illness*	Any value	Treat
HIV-related Symptomatic**	Any value	Treat
Asymptomatic	<350	Treat
Asymptomatic	≥350	Defer treatment; follow up clinical status and monitor CD4+ T-cell count every 6 months
Pregnancy	Any value	Treat, Discontinue ART after delivery if pre-treatment CD4+ T-cell count is ≥350 cells/mm^3

Table 3.6 Recommended second-line ART regimens for patients with treatment failure

First regimens	Recommended second regimens
2 NRTIs + NNRTI	Boosted PI + 2 active NRTIs indicated by genotype testing
2 NRTIs + boosted PI	1) active boosted PI + 2 active NRTIs indicated by genotype testing 2) active boosted PI + 1 NNRTI ± 1 NRTI indicated by genotype testing 3) NNRTI + 2 active NRTIs indicated by genotype testing This option can be used only when there are 2 active NRTIs and the patient has never been exposed to NNRTI, or NRTI monotherapy or duotherapy.

To prevent mother-to-child transmission (MTCT) of HIV, the guidelines are divided into 2 main aspects, treatment in HIV-infected pregnant women who have

not been on antiretroviral treatment prior to pregnancy and treatment in HIV-infected pregnant women who conceive while on highly active antiretroviral therapy (HAART). For previously untreated prior to pregnancy women, a lopinavir/ritonavir (LPV/r)-based HAART is recommended since infected pregnancy women who have CD4 count ≥250 cells/mm^3 may develop NVP-associated hepatotoxicity. In case of previously treated HIV-infected pregnant women, the efficacy of the current HAART regimen should be concerned. The best HAART regimen should provide an undetectable plasma HIV-1 RNA level. The treatment failure is indicated with plasma HIV-1 RNA levels ≥1,000 copies/ml after adhering with the current HAART for more than six months, in that case an HIV specialist should be consulted. If the current HAART regimen is successful and does not contain AZT or the HIV-infected pregnant women do not experience with resistance or severe side effects from AZT, one of the nucleoside reverse transcriptase inhibitors used should be replace with AZT. As the woman in the first trimester is not recommending for EFV treatment, the current HAART regimen that contains EFV should be replaced with LPV/r.

3.6 HIV drug resistance mutations (Table 3.7)

HIV drug resistance was developed because of two reasons. First, HIV infection have high levels of virus production and turnover. HIV infection can estimate number of productively infected cells in lymphoid tissue about 10^7 to 10^8 cells. During chronic phase, this number is stable that the balance new target cells and their clearance. Because they have short half life (1-2 days) so this state requires a very high rate. Second the viral population in an infected person is highly heterogeneous. So drug resistance mutants are present prior to initiation drug therapy. The reverse transcription of viral RNA into DNA that has error rates estimated at 1 error per 1-30,000. Most of these errors are base substitutions, but duplications, insertions, and recombination can also occur.

The significantly increase of antiretroviral therapy (ART) led to the development of viral resistance. Regarding to the study of Sanguansittianant et al, 369 samples from HIV-1-infected subjects in Thailand during 2009-2010 associated with 61% resistance to nucleoside analogue reverse transcriptase inhibitors (NRTI's),

64.2% in nonnucleoside analogue reverse transcriptase inhibitors (NNRTI's), and a total of 49.6% was found in combined NRTI and NNRTI (50). ART-naïve Thai patients who had ART initiation between 2010 and 2011 showed a drug resistance-associated mutations (DRAMs) prevalence of 17% for NNRTIs, 0.6% for NRTIs and 0.6% for protease inhibitors (PIs) (51). In addition, Thailand has been reported as the most first-line treatment failure in Sounteast Asia with 89-95% resistance of NNRTI and 42-58% resistance of NRTI in adults (52). In conclusion, NNRTIs have been shown the higher resistance prevalence than NRTIs and PIs in HIV-1-infected patient in Thailand.

Table 3.7 HIV-1 drug resistance mutations. (24)

Mutation	Comments
Reverse transcriptase	
Mutation conferring resistance to nucleoside analogues	Family of mutations known as thymidine analogue mutations. Associated with resistance to most nucleoside analogues except lamivudine.
M41L D67N	In vitro, cause high level resistance to zidovudine and low level resistance to stavudine, didanosine and abacavir.
K70R L210W	Segregate in two pathways, one comprising T215Y and L210W and the other T215F and K219Q.
T215Y, T215F K219Q, K219E	Pathway comprising T215Y and L210W associated with decreased responsiveness to tenofovir.
M184V	Observed in most viruses resistant to treatment with lamivudine. Confers high level resistance to lamivudine in vitro. Can interfere with resistance to zidovudine and stavudine when number of thymidine analogue mutation is small. Increases the level resistance to didanosine and abacavir owing thymidine analogue mutations.
Q151M	Rare pathway for resistance of HIV-1 to nucleoside analogue.
F116Y F77L V75I A52V	In vitro, causes high level resistance to most nucleoside analogues except lamivudine and tenofovir.
69 insertions mutations	Insertions of 2 or more amino acids (usually serines) next to codon 69. Emerge only viruses that already have several thymidine analogues mutations. Confer high level resistance to all nucleoside analogue.
K65R	Selected for by zalcitabine, abacavir and tenofovir therapy
Y115F	Selected for by abacavir therapy.
L74V	Selected for by didanosine therapy usually when didanosine is the only nucleoside analogue.
Mutations conferring resistance to NNRTIs	
K103N	Mutations most frequency selected for by efavirenz therapy. Occasionally selected for by nevirapine therapy. Confers high level resistance to all available NNRTIs.
Y181C Y188C V108I	Mutations most frequently selected by nevaripine. Confers high level resistance to nevaripine but lower level resistance to efavirenze. Y188L unlike Y188C seen mostly with efavirenz therapy.

Table 3.7 HIV-1 drug resistance mutations. (cont.)

Mutations conferring resistance to NNRTIs	
L100I V106A G190A, G190S	Mutations that accumulate during prolonged ineffective therapy with most NNRTIs
Mutation	**Comments**
Protease and gag mutations	
L90M	Frequent resistance mutation, observed during failure of therapy with most protease inhibitors.
V82A,V82I, V82F	Common resistance mutations. Can emerge early during failure of therapy with most protease inhibitors. Mutations most frequently for by ritonavir and indinavir therapy.
D30N N88D, N88S	Mutations most frequently selected for by nelfinavir therapy. D30N always first.
L10I, L10F K20R, K20M M36I M46I, M46L I54V, I54L A71V, A71T G73S V77I M93L	Mutations that can accumulate during failure of therapy with most protease inhibitors, causing gradual increases in the level of resistance.
I84V	Frequently found after prolonged ineffective therapy with protease inhibitors. Associated with high level resistance to most protease inhibitors.
G48V	Exclusive selected for by saquinavir therapy. Associated with high level resistance to saquinavir.
L24I	Emerges occasionally during failure of indinavir therapy. Also found with lopinavir therapy.
I47V, I50V	Most often selected for by amprenavir therapy. Also found with lopinavir therapy.
V32I, F53L	Rare mutations. Confer high level resistance most protease inhibitors.
A431V L449F	Mutations in gag the main viral substrate of the protease. Increase resistance and partially compensate for resistance associated loss of viral replicative capacity.

CHAPTER IV
MATERIALS AND METHODS

4.1 Subject

4.1.1 Ethical consideration

Mahidol University Institutional Review Board (MU-IRB) Mahidol University, Thailand approve the study protocol. Ethical clearance for the use of patient sample material in this study was obtained from Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand (ethics certificate of approval number: 304/2555(EC3)).

4.1.2 Study population

4.1.2.1 Subjects

A total of 675 EDTA blood samples from patients who attended for antiretroviral therapy from 5 hospitals representing Central, Southern, Northeastern and Northern regions; Siriraj hospital, Sawanpracharak hospital, Sappasittiparasong hospital and Suratthani hospital and the office of Disease Prevention and Control 12 Songkhla that sent for HIV drug resistant genotype detection at Department of Microbiology Siriraj Hospital during January 2009 to December 2011 were collected. According to sample size calculation, 35 random samples of the collected samples from 2009 (n=111), 2010 (n=250) and 2011 (n=314). The demographic data of these study population are shown in tables 4.1 - 4.3.

Table 4.1 Demographic characteristics of 675 EDTA blood samples.

Year isolated	2009	2010	2011
Amount of sample	111	250	314
Average age	33.6	35.6	33.0
Male (%)	44.1	55.4	65.7
Female (%)	55.9	44.6	34.3
Viral load log^{10} (copies/ml)	4.90	4.95	5.10

Table 4.2 Average HIV viral load of 675 EDTA blood samples

Viral load (copies/mL)	2009	2010	2011
<2000	1	14	21
2,000-10,000	44	104	133
10,000-50,000	29	60	75
50,000-100,000	12	41	45
>100000	21	30	40
no data	4	1	-

Table 4.3 Demographic characteristics of 35 random samples.

Year isolated	2009	2010	2011
Amount sample	6	7	22
Average age	29.33	28.57	31.63
Gender			
Male (%)	33.33(2/6)	71.43(5/7)	54.54(12/22)
Female (%)	66.67(4/6)	28.57(2/7)	45.46(10/22)
Viral load log 10 (copies/ml)	5.14	4.11	4.75
Mean CD4 counts (cell/mm^3)	191.9	276.84	382.97

4.2 Sample collection and processing

RNAs were extracted according to Viroseq HIV-1 drug resistant kit. Briefly, 500 µl of plasma will be centrifuged at 21,000-25,000 g, 4^{o}C for at least 1 hour. During centrifugation, HIV Viral Lysis Buffer will be thawed at room temperature. If the buffer contains some crystals, incubate at 37^{o}C until the precipitate disappears then bring to room temperature. Thaw RNA diluent at 2^{o}C-8^{o}C, then, prepare 70% alcohol 1 ml for each sample and keep in refrigerator at 2^{o}C-8^{o}C. After centrifugation, discard aqueous phase and resuspend pellet with 600 µl of HIV Viral Lysis Buffer. Mix thoroughly for 5-10 seconds and incubate for 10 minutes at room temperature. Add 600 µl of isopropanol follow by centrifuge at 12,500-15,000 g for 15 minutes. Remove supernatant and add 1 ml of cold 70% alcohol. Mix thoroughly and centrifuge at 12,500-15,000 g for 5 minutes at 4^{o}C. Remove the supernatant as much as possible and centrifuge 8,000 rpm for1 minute. Remove the leftover supernatant by pipetting and dry the pellet at room temperature for 5 minutes or until all 70% ethanol be evaporated. Add RNA diluent as following table.

HIV viral load	**RNA diluent volume (µl)**
More than 15,000 copies/ml	100
2,000–15,000 copies/ml	50
Unknown	50

Finally, resuspend pellet with RNA diluent by agitation about 10-20 seconds then centrifuge at 8,000 rpm for 10 seconds and keep at -80^{o}C.

4.2.1 Sample size calculation

The proper sample size will be calculated based on a simple random sample, which can be calculated according to the following formula

$$n = Z_{\alpha/2}^2 pq/d^2$$

n	refers to sample size in each year
$Z_{\alpha/2}^2$	refers to a confidence interval which is 1.96
p	refers to an estimated proportion
q	refers to 1-p
d	refers to an errors in estimating the proportion

4.3 HIV viral load determination

HIV-1 RNA quantitation(HIV-VL) in plasma samples were determined by using HIV-1 viral load assay Abbott m2000 Real Time HIV-1 (Applied Biosystems, Foster city, California, USA), USA). Sample preparation, nucleic acid extraction for amplification and detection were done following the manufacturer's protocol

Briefly, the m2000 Real Time HIV-1 assay combines an automated extraction (input volume of 0.6 ml, m2000sp apparatus), a real-time PCR amplification of the *integrase* gene fragment and a noncompetitive fluorescent detection (m2000rt instrument, dynamic range of 40 to 10^7 copies/ml).

4.4 HIV-1 nucleotide sequence analysis

4.4.1 Amplification of HIV-1 *gag* gene

cDNA of HIV-1 *gag* gene will be generated by using Superscript III reverse transcriptase (Invitrogen, USA). cDNA will be amplified with PrimeSTAR GXL DNA polymerase (Takara, Japan) by nested PCR with two primer set as shown in Table 4.4.

Table 4.4 List of primers for HIV-1 *gag* amplification (1)

Primer	Location*	Primer sequence (5'-3')
msf12b	623→649	AAATCTCTAGCAGTGGCGCCCGAACAG
RT3474R	3453→3477	GAATCTCTCTGTTTTCTGCCAGTTC
f2nst	769→793	GCGGAGGCTAGAAGGAGAGAGATGG
SP3AS	2382→2407	CCTCCAATTCCCCCTATCATTTTTGG

* Numbering of nucleotide is related to HXB2

cDNA will be synthesized from 10 µl of extracted RNA in first master mix which contains 1 µl of 10mM dNTP, 0.8 µl of RT3474R primer (100 pmol/ µl), 1.2 µl DNase free water. Heat reaction at 65^{o}C for 5 minutes and place on ice at least 1 minute. Add 7 µl of second master mix which contains 4 µl 5X first strand buffer, 1 µl 0.1M DTT, 1 µl RNase Out(400 U) (Invitrogen, USA) and 1 µl Superscript III reverse transcriptase (Invitrogen, USA). Incubate at 50^{o}C for 60 minutes, follow by 70^{o}C for 15 minutes to stop activity of reverse transcriptase enzyme and then add 1 µl RNase H and incubate at 37^{o}C for 20 minutes to destroy RNA template. The size of cDNA is 3474 base pair.

For first round PCR, 0.2 µl each of RT347R primer (20 pmol/ µl) and f2nst (20 pmol/ µl) will be used to amplified *gag* gene in reaction mix of 4 µl 5X primeSTAR GXL, 1.6 µl dNTP mixture (2.5 mM each), 0.4µl primeSTAR GXL DNA pol (1.25 U/µL) (Takara, Japan), 11.6 µl DNase free water. 2 µl cDNA will be added to reaction and place on thermal cycler 9700 (Perkin, USA). The amplification cycle will be programmed as following; 35 cycles of 98^{o}C for 10 seconds, 55^{o}C for 15 seconds, 68^{o}C for 3 minutes and 1 cycle of 68^{o}C for 10 minutes.

For nested PCR, 1 µl each of f2nst primer (20 pmol/ µl) and1 µl SP3AS primer (20 µl), will be used in reaction mix of 10 µl 5X primeSTAR GXL, 4 µl dNTP mixture (2.5 mM each), 1µl primeSTAR GXL DNA pol (1.25 U/µL) (Takara, Japan) and 28 µl DNase free water. Five microliters of PCR product from first round will be added to reaction and place on thermal cycler 9700 (Perkin, USA). The amplification cycle will be programmed as following; 35 cycles of 98ºC for 10 seconds, 55ºC for 15 seconds, 68ºC for 3 minutes and 1 cycle of 68ºC for 10 minutes. The amplified product of HIV-1 *gag pol* is 1600 base pairs.

4.4.2 Amplification of HIV-1 *pol* gene by viroseq (Celera, USA)

HIV-1 *gag* gene will be generated by using Viroseq HIV-1kit

Table 4.5 Master mix of Viroseq HIV-1 kit

Master mix	1 Reaction (µl)
HIV RT mix	8
Rnase Inhibitor	1
MuLV Reverse Transcriptase	1
DTT 100 mM	0.4
Final volume	10.4

cDNA will be synthesized from 10 µl of extracted RNA. Incubate RNA at 65ºC for 0.5 minutes, follow by 42ºC for 5 minutes and stop machine. Add master mix (Table 4.5) 10 µl and incubate at 42ºC for 60 minutes, follow by 42ºC for 5 minutes.

For PCR, 29.5 µl each of HIV PCR mix, 0.5 µl of AmpliTaq Gold polymerase, 1 µl of AmpErase UNG. Add 30 µl PCR mix in cDNA tube and place on thermal cycler 9700 (Perkin, USA). The amplification cycle will be programmed as following; 35 cycles of 98ºC for 10 seconds, 55ºC for 15 seconds, 68ºC for 3 minutes and 1 cycle of 68ºC for 10 minutes.

4.4.3 Amplification of HIV-1 *env* gene regions

Table 4.6. List of primers for HIV-1 *env* amplification (48)

Primer	**Location***	**Oligonucleotide sequences (5'-3')**
1R3B3R	9642→9611	ACTACTTGAAGCACTCAAGGCAAGCTTTATTG
07for7	4875→4912	CAAATTAYAAAAATTCAAAATTTTCGGGTTTA TTACAG
2.R3.B6 R	9607→9636	TGAAGCACTCAAGGCAAGCTTTATTGAGGC
VIF 1	4900→4923	GGGTTTATTACAGGGACAGCAGAG
Low2C	9591→9612	TGAGGCTTAAGCAGTGGGTTCC

*****Numbering of nucleotide is related to HXB2

All primers used for HIV-1 env amplifind was showed in Table 4.6. cDNA will be synthesized from 10 µl of extracted RNA in first mastermix which contains 1 µl of 10mM dNTP, 0.8 µl of 1R3B3R primer (100 pmol/ µl), 1.2 µl DNase free water. Heat reaction at 65ºC for 5 minutes and place on ice at least 1 minute. Add 7 µl of second master mix which contains 4 µl 5X first strand buffer, 1 µl 0.1M DTT, 1 µl RNase Out (400 U) (Invitrogen, USA) and 1 µl Superscript III reverse transcriptase (Invitrogen, USA). Incubate at 50ºC for 60 minutes, followed by 70ºC for 15 minutes to stop activity of reverse transcriptase enzyme and then add 1 µl RNase H and incubate at 37ºC for 20 minutes to destroy RNA template. The size of cDNA is 4500 base pairs.

For the first round PCR, 0.2 µl each of 07for7 primer (20 pmol/ µl) and 2R3B6R (20 pmol/ µl) will be used to amplified *env* gene in reaction mix of 4 µl 5X primeSTAR GXL, 1.6 µl dNTP mixture (2.5 mM each), 0.4 µl primeSTAR GXL DNA pol (1.25 U/µL) (Takara, Japan), 11.6 µl DNase free water. 2 µl cDNA will be added to reaction and place on thermal cycler 9700 (Perkin, USA). The amplification cycle will be programmed as following; 35 cycles of 98ºC for 10 seconds, 60ºC for 15 seconds, 68ºC for 4 minutes and 1 cycle of 68ºC for 10 minutes.

For nested PCR, 1 µl each of Low2C primer (20 pmol/ µl) and1 µl VIF 1 primer (20 µl), will be used in reaction mix of 10 µl 5X primeSTAR GXL, 4 µl dNTP mixture (2.5 mM each), 1µl primeSTAR GXL DNA pol (1.25 U/µL) (Takara, Japan)

and 28 µl DNase free water. Five microliters of PCR product from first round will be added to reaction and place on thermal cycler 9700 (Perkin, USA). The amplification cycle will be programmed as following; 35 cycles of 98ºC for 10 seconds, 55ºC for 15 seconds, 68ºC for 3 minutes and 1 cycle of 68ºC for 10 minutes. The amplified product of HIV-1 *gag env* is 4760 base pairs.

4.4.4 PCR product detection and purification

The amplified PCR products will be detect by gel electrophoresis. 1% of agarose gel will be used to detect DNA fragment of HIV-1 *env, gag* and *pol* gene. Briefly, one grams of agarose gel (Biolin, Spain) will be dissolved in 0.5XTBE (Tris-borate EDTA) and boiled until gel will be completely melted. Place at room temperature to cool down and pour gel in casting platform with gel comb. Leave gel at room temperature until gel will be set. Take comb off and place gel into chamber contained 0.5XTBE buffer. Load PCR product into well by mixing 5 µl of PCR product with 6X loading dye (Fermentus, EU). The electrophoresis will be run at constant 150 Voltages for 1 hour or until dye marker will be on the bottom of gel. Place gel in Gel-Red (Biotium, USA) for 5-10 minutes. The fragment will be observed under UV transluminator. The DNA fragment of *gag*, *env* and *pol* genes will be 1600, 4760 and 3748 bp, respectively.

To clean up PCR product, PureLink® PCR Purification Kit (Invitrogen, USA) will be used. Briefly, 4 volumes of Binding Buffer HC (B3) and 1 volume of isopropanol will be added to PCR product and mixed. The mixture will be pipetted into a PureLink Spin column with a collection tube and centrifuged the column at > 10,000 g for 1 minute. Then, the column will be reinserted into the collection tube and added 650 µl wash buffer (W1). Centrifuge the column at >10,000 g for 1 minute and discard the flow through. The column will be placed in the same collection tube and centrifuged at maximum speed for 3 minutes. Later, the column will be placed into a clean 1.7 ml elution tube then add 50 µl elution buffer to the center of the column and incubate the column at room temperature for 1 minute. Finally, the column will be centrifuged at maximum speed for 2 minutes. The purified DNA will be kept at -20ºC for long term storage.

4.4.5 DNA concentration determination

Prior to start sequencing step, PCR product will be determined concentration by using Nanodrop spectrophotometer. Two microliters of PCR product will be used for measurement. The absorbance at 260 will be measured and convert to concentration in terms of ng/µl. The requirement ratio of PCR product purity will be more than 1.70 (A260/A280) and the quantity will be accepted 20-100 ng/µl.

4.4.6 HIV-1 *pol* gene sequencing

Protease and RT regions will be sequenced according to Viroseq HIV-1 kit (Celera, USA). One sample will compose of 7 primer sequencings (A, B, C, D, F, G and H). Briefly, 12 µl of each HIV SEQ MIX reagents (A, B, C, D, F, G and H primer sequencings) will be added to Microamp 96 well plate followed by adding 8 µl of diluted sample into wells and place plate to Thermal cycle 9700 machine. Set the amplification cycle as follow; starting with 25 cycles of 96^{o}C for 10 seconds, 50^{o}C for 5 seconds, 60^{o}C for 4 minutes. Keep plate at -20^{o}C until precipitation step.

4.4.7 HIV-1 *gag* and *env* gene sequencing

Oligonucleotide sequencing will be performed with Sanger method. The procedure will compose of three steps, incorporation of the target gene, extraction of the extension product and loading the extension product. HIV-1 *gag* and *env* gene will be sequenced by using Bigdye Terminator Cycle Sequencing ready reaction version 3.1(ABI PRISM, Perkin Elmer, Foster city, USA). Briefly, add 4 µl of 5X Bigdye buffer, 3.2 pmol of primer, 4 µl of Bigdye terminator version 3.1, 100-500 ng/µl of purified PCR product and add DNase free water until 20 µl. Set program for cycle sequencing as following: 96^{o}C for 3 minutes and 35 cycles of 96^{o}C for 10 seconds, 50^{o}C for 5 seconds, 60^{o}C for 2 minutes. Keep plate at -20^{o}C until precipitation step.

4.4.8 Purification sequencing reaction

To remove excess dyes from sequencing reaction, ethanol precipitation will be used for purification step. Briefly, 60 µl of isopropanol and 20 µl DNaseRnase free water will be added to each reaction. Incubate plate for 15 minutes at room temperature in dark. Centrifuge at 2,000 g for at least 45 minutes and remove solution

by inverting plate on tissues and centrifuge at 700 g for 1 minute. Pellet will be resuspended by adding 20 µl of Hi Di formamide to each well and mixed. Centrifuge at 700 g for 10-20 seconds. Plate will be covered with rubber septa after denaturing at 95ºC for 2 minutes. Place a plate to 3130 Genetic Analyzers machine for sequencing.

4.4.9 HIV-1 Antiretroviral Drug Resistance Analysis

HIV-1 *pol* gene nucleotide sequence were anlyzed by using Viroseq software version 2.8 (Celera, USA) and HIVdb program in the HIV Drug Resistance Database from Stanford University (http://hivdb.stanford.edu/)

4.4.10 Nucleotide sequence analysis

Ab1. data file acquired from Genetic Analyzer were imported into the DNA BASER and assembled into contiguous fragments. The nucleotide sequences were finally exported as FASTA file format for further analysis.

Dataset containing sample nucleotide sequences and all reference subtypes sequences from Los Alamos sequence database were analyzed. The Molecular Evolutionary Genetics Analysis (MEGA) software version 7 was used for phylogenetic analyses. Phylogenetic trees were constructed using Maximum Likelihood method based on the Tamura-Nei model.

HIV-1 genomes recombinance detection uses jpHMM (jumping profile Hidden Markov Model) web tool. It aligns a query sequence to pre-calculate multiple alignments of the major HIV-1 subtypes, to predict recombination breakpoints in each region of the sequence assigned.

RIP (Recombination identification program) tool was also used to identify recombination in query sequence by calculating similarity to a background alignment in a sliding window. The graphical output from the program shows the different distance measurement between the query and various background sequences.

REGA HIV-1 Subtyping Tool. This tool is designed to use phylogenetic methods in order to identify the subtype of a specific sequence. The sequence is analysed for recombination using bootscanning methods.

4.4.11 Construction of Phylogenetic Tree Using MEGA 7

After the manual inspection of alignments, Molecular Evolutionary Genetics Analysis (MEGA) software version 7 was used for phylogenetic analyses. Phylogenetic trees were constructed Maximum Likelihood method based on the Tamura-Nei model.

Adjusted multiple alignment files performed by MUSCLE were imported into MEGA v 7 where the alignment files saved in (.mas) format were converted into MEGA format (.meg). The MEGA files (.meg) were then opened in MEGA and Maximum Likelihood were constructed with the use of the Kimura 2 parameter method of nucleotide substitution.

CHAPTER V
RESULTS

5.1 Study population characterization

A total of 675 EDTA blood samples from HIV infected patients sent to Department of Microbiology for HIV drug resistant genotype assays year 2009-2011 were used in this study. HIV drug resistant genotype assay was performed by using Viroseq genotype assay. The samples can be divided according to year of collection as 2009 (n=111), 2010 (n=250) and 2011 (n=314). Demographic characteristics of a total of 675 EDTA blood samples were showed in Tables 4.1 – 4.3 and figure 5.1

In 2009, 33, 39, 35 and 35 samples were collected from Bangkok, Nakhonsawan, Suratthani and Songkhla, respectively. The average age of 2009 subjectswas 33 years and 44.1% and 55.9% of them were male and female. The average of viral load and CD4 value of 2009 samples were log 4.90 copies/ml and 191.9cell/mm^3.

In 2010, 12, 196, 31 and 11 samples were collected from Bangkok, Nakhonsawan, Suratthani and Ubon Ratchathani, respectively. The average age of subjects was 35.6 years and 55.4% and 44.6% of them were male and female. The average of viral load and CD4 value of 2010 samples were log 4.95 copies/ml and 276.84 cell/mm^3.

In 2011, 19, 184, 7, 15 and 89 samples were collected from Bangkok, Nakhonsawan, Suratthani, Songkhla and Ubon Ratchathani, respectively. The average age of subjects was 33 years and 65.7%and 34.3% of them were male and female.The average of viral load and CD4 value of 2011 samples were log 5.10 copies/ml and 382.97 cell/mm^3.

Of 675 samples, there were 610 (90.37%) samples, who received first line drug regimen (GPO-VIR), 60(8.89%) samples received other NRTIs + NNRTI antiretroviral drug and 5(0.74%) samples with unknown antiretroviral drugs.

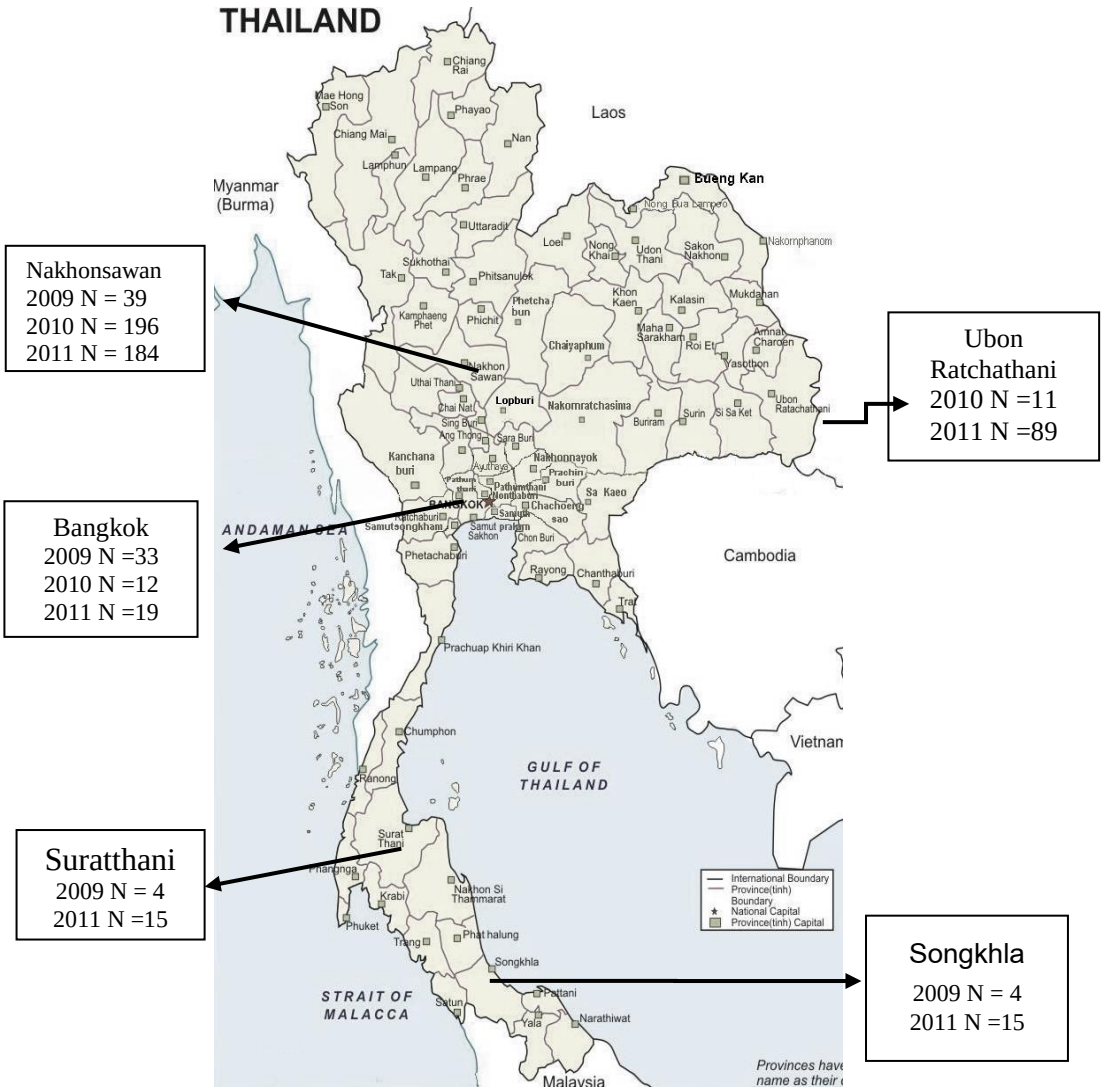


Figure 5.1 Location of sample collection in the study

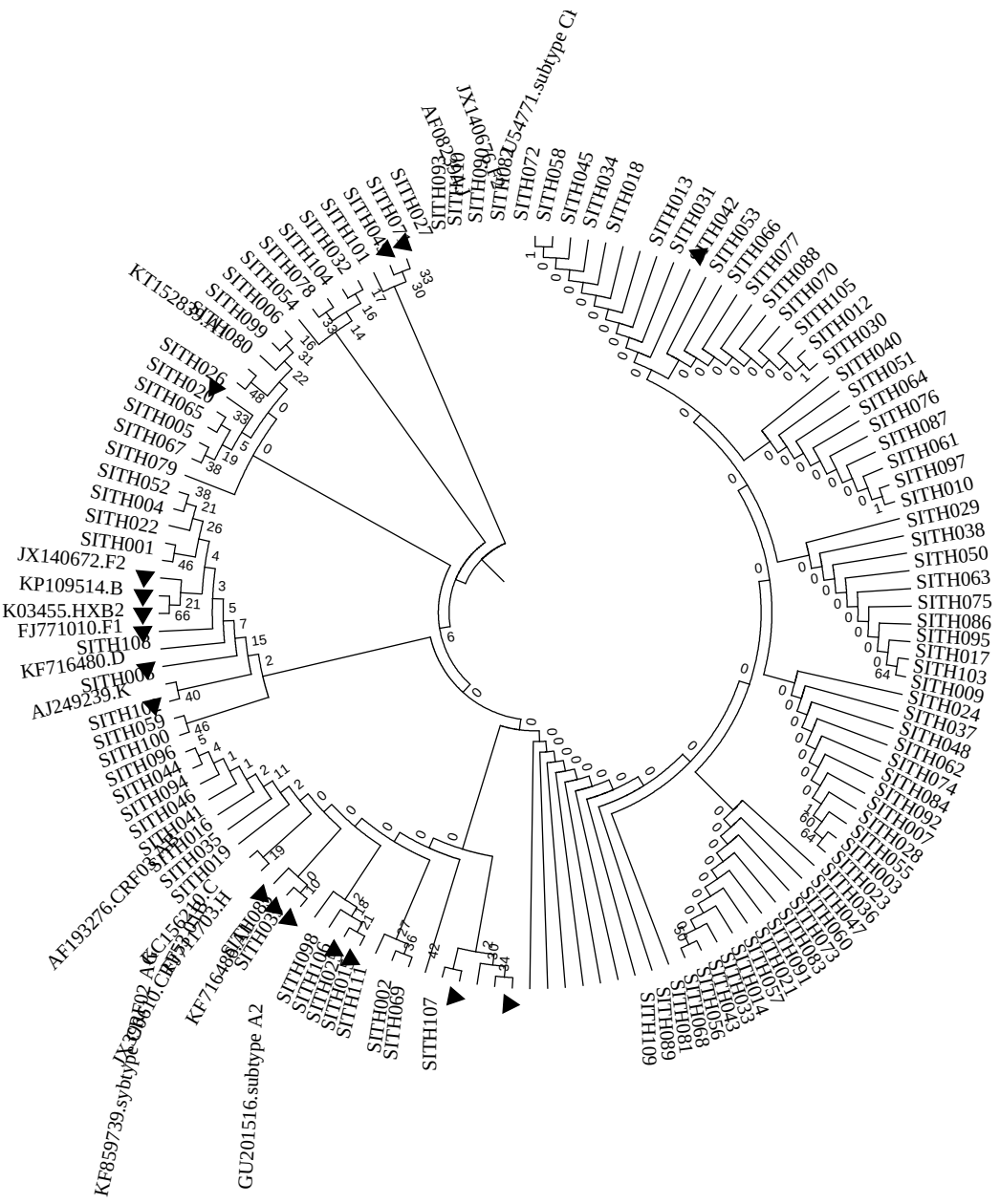
5.2 HIV- 1 subtype determination by *pol* nucleotide sequence data from drug resistant genotyping

HIV *pol* nucleotide sequences [reverse transcriptase (RT) and protease (PR) region] resulted from Viroseq genotyping assay were submitted to Standford database for drug resistant mutations (DRMs) determination and HIV subtyping. These HIV subtype data was compared with phylogenetic analysis of RT and PR nucleotide sequences, figures 5.2-5.4. The result of HIV subtype analyzed by Stanford HIV database and phylogenetic analysis were shown in tables 5.1 and 5.2. HIV CRF01_AE was circulating predominantly in Thailand during 2009-2011. Moreover,

HIV subtype B was secondly found and followed by others subtype and circulating recombinant strains including CRF02_AG and subtype C.

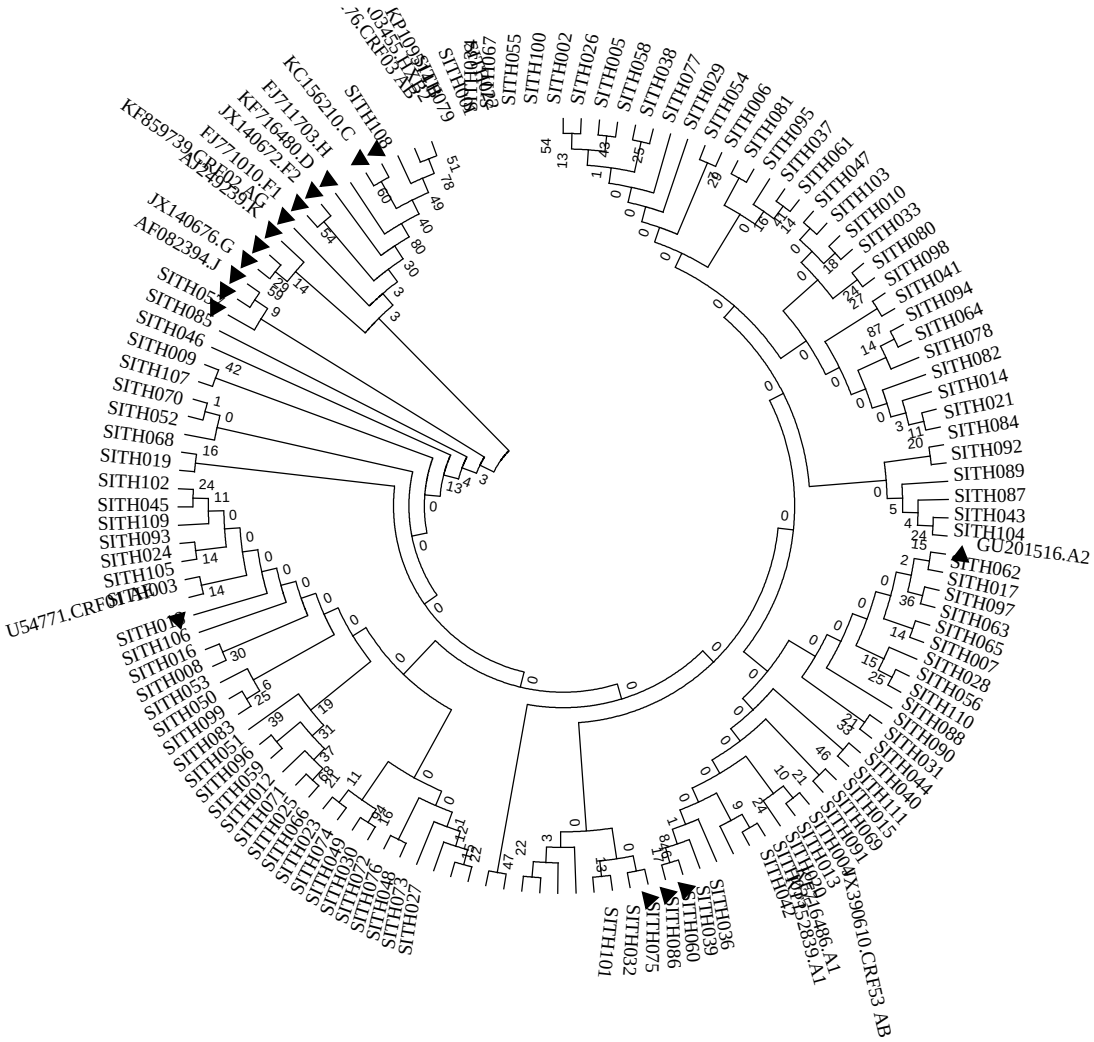
Table 5.1 Percentage of HIV-1 subtypes found in 675 samples analyzed by Stanford HIV database (69) of RT and PR nucleotide sequences

HIV subtype	2009	2010	2011
B	3.6%(4/111)	4%(10/250)	3.8%(12/314)
CRF01_AE	94.6%(105/111)	93.2%(233/250)	93.9%(295/314)
C	0%(0/111)	0%(0/250)	0.3%(1/314)
CRF01_AE/B	1.8%(2/111)	0.8%(2/250)	0.6%(2/314)
D/CRF01_AE	0	0.8%(2/250)	0
CRF02AG/CRF01AE	0	0.4(1/250)	0
K/CRF01_AE	0	0.8%(2/250)	0.6%(2/314)
D/B	0	0	0.3(1/314)



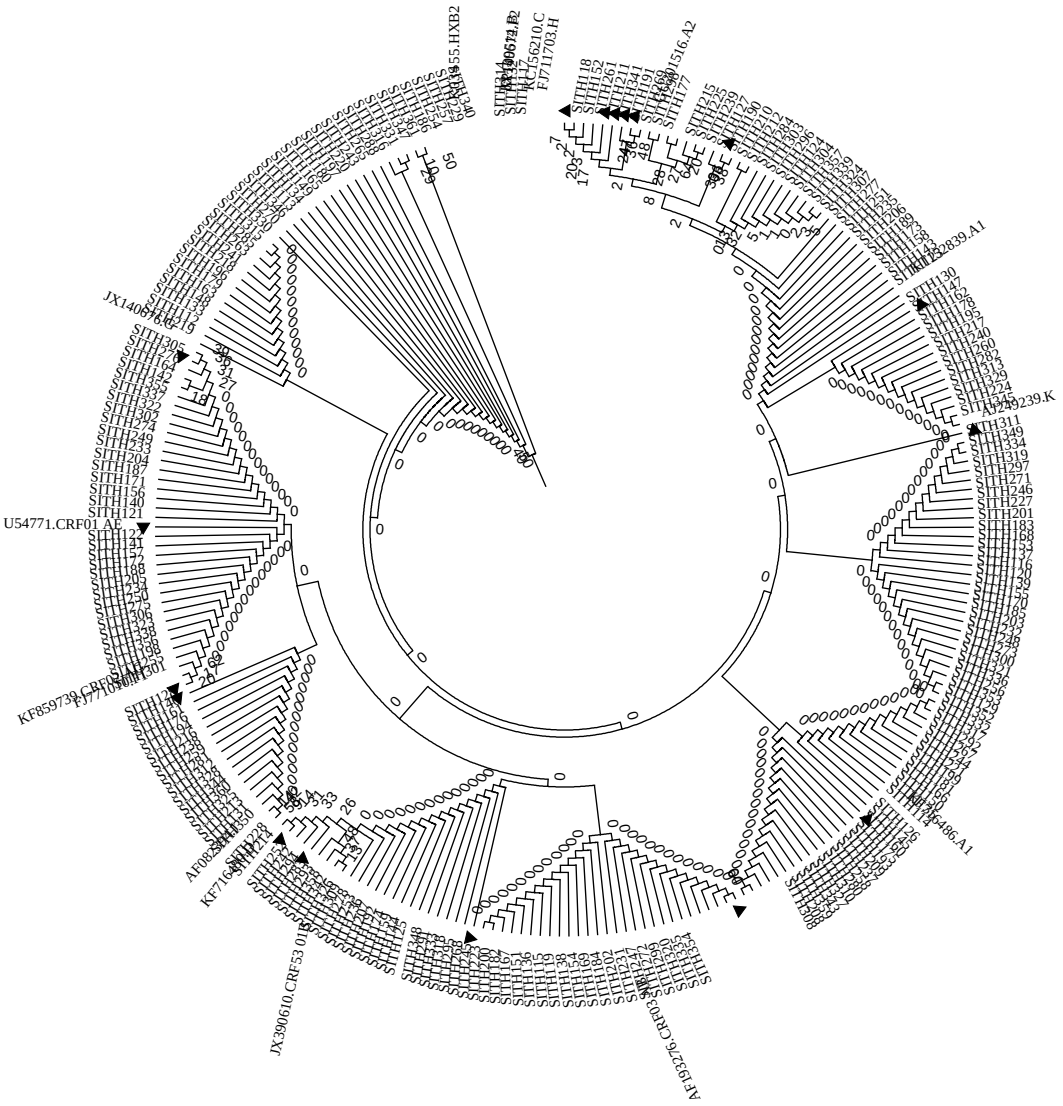
(a)

Figure 5.2 Neighbor-joining tree analysis of (a) PR nucleotide sequences and (b) RT nucleotide sequences of 111 samples collected in 2009. Labels filled triangle show HIV-1 subtype reference sequences.



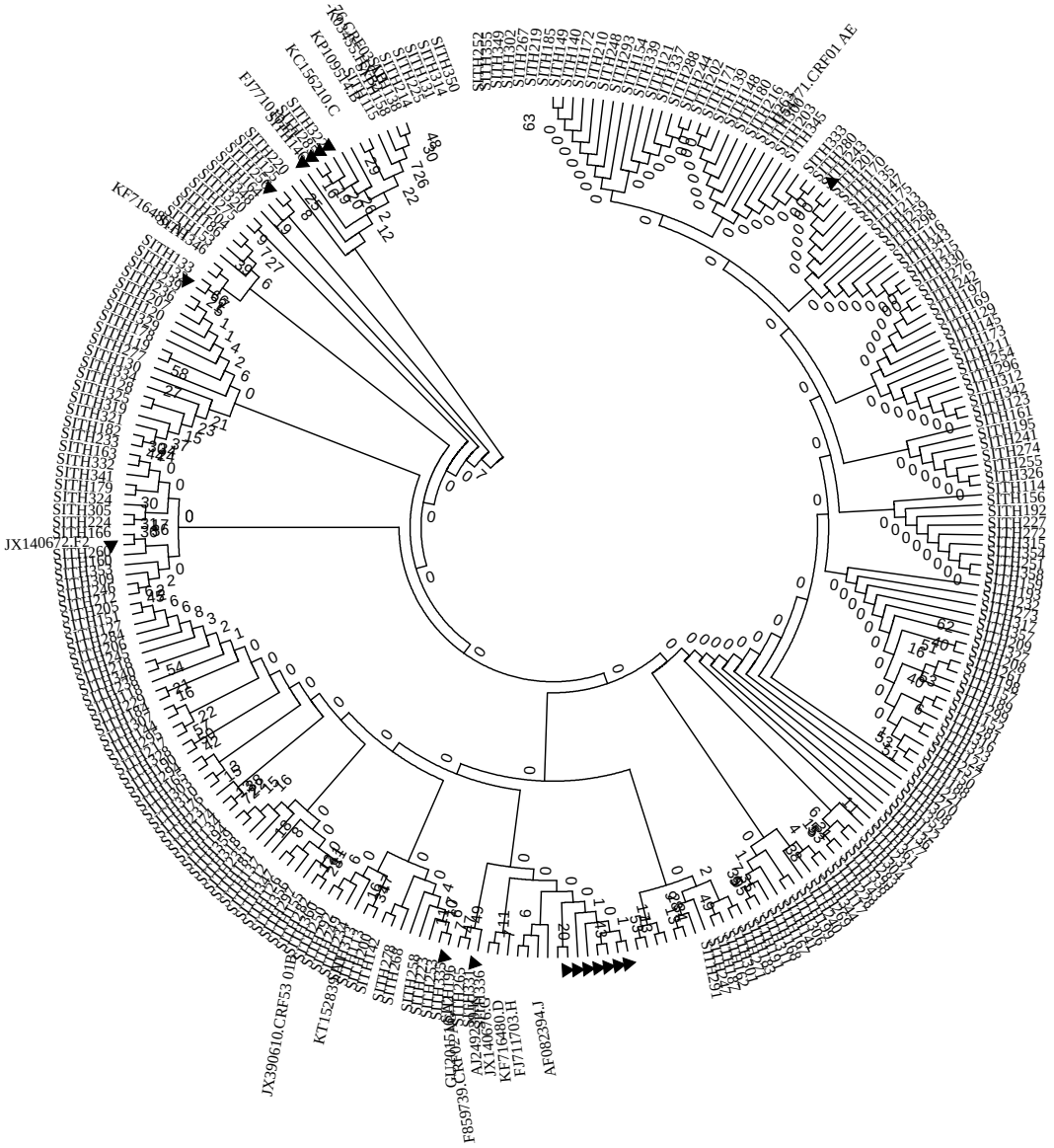
(b)

Figure 5.2 Neighbor-joining tree analysis of (a) PR nucleotide sequences and (b) RT nucleotide sequences of 111 samples collected in 2009. Labels filled triangle show HIV-1 subtype reference sequences (cont.).



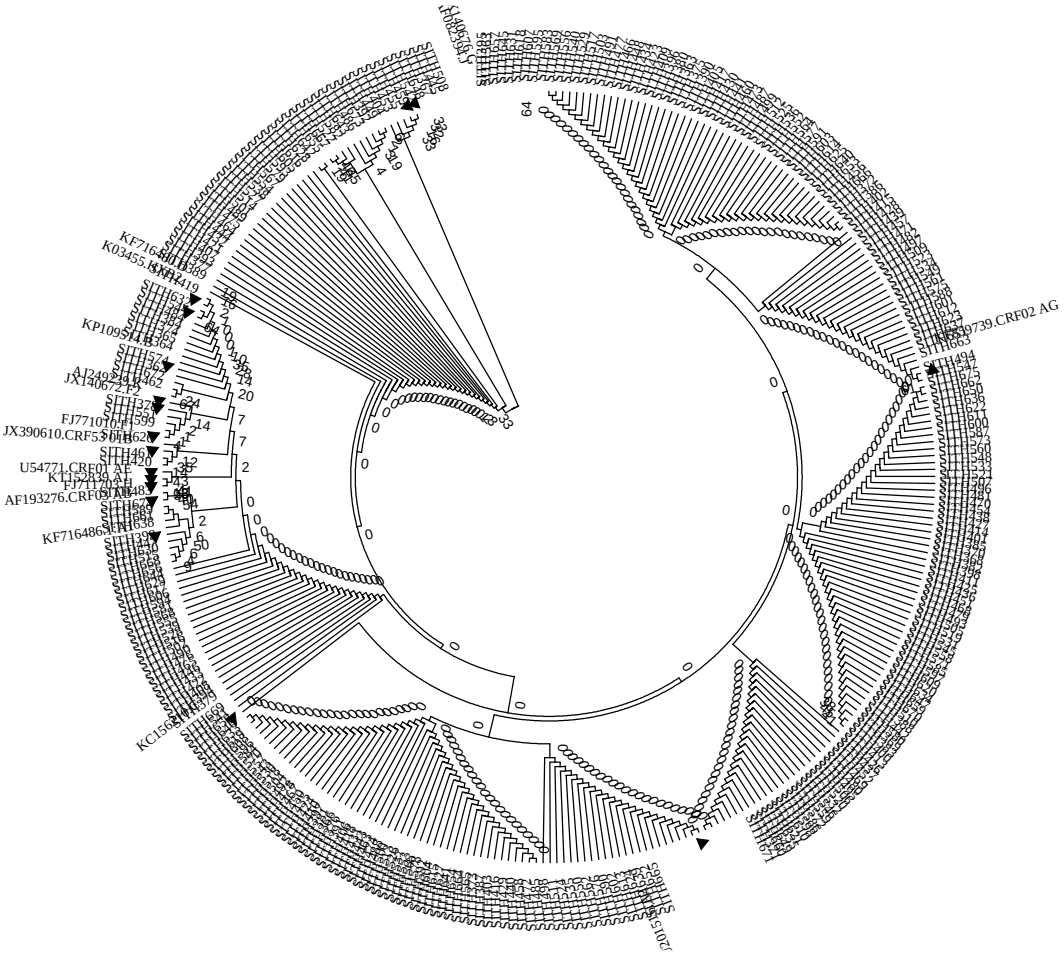
(a)

Figure 5.3 Neighbor-joining tree analysis of (a) RT nucleotide sequences and (b) PR nucleotide sequences of 250 samples collected in 2010. Labels filled triangle show HIV-1 subtype reference sequences.



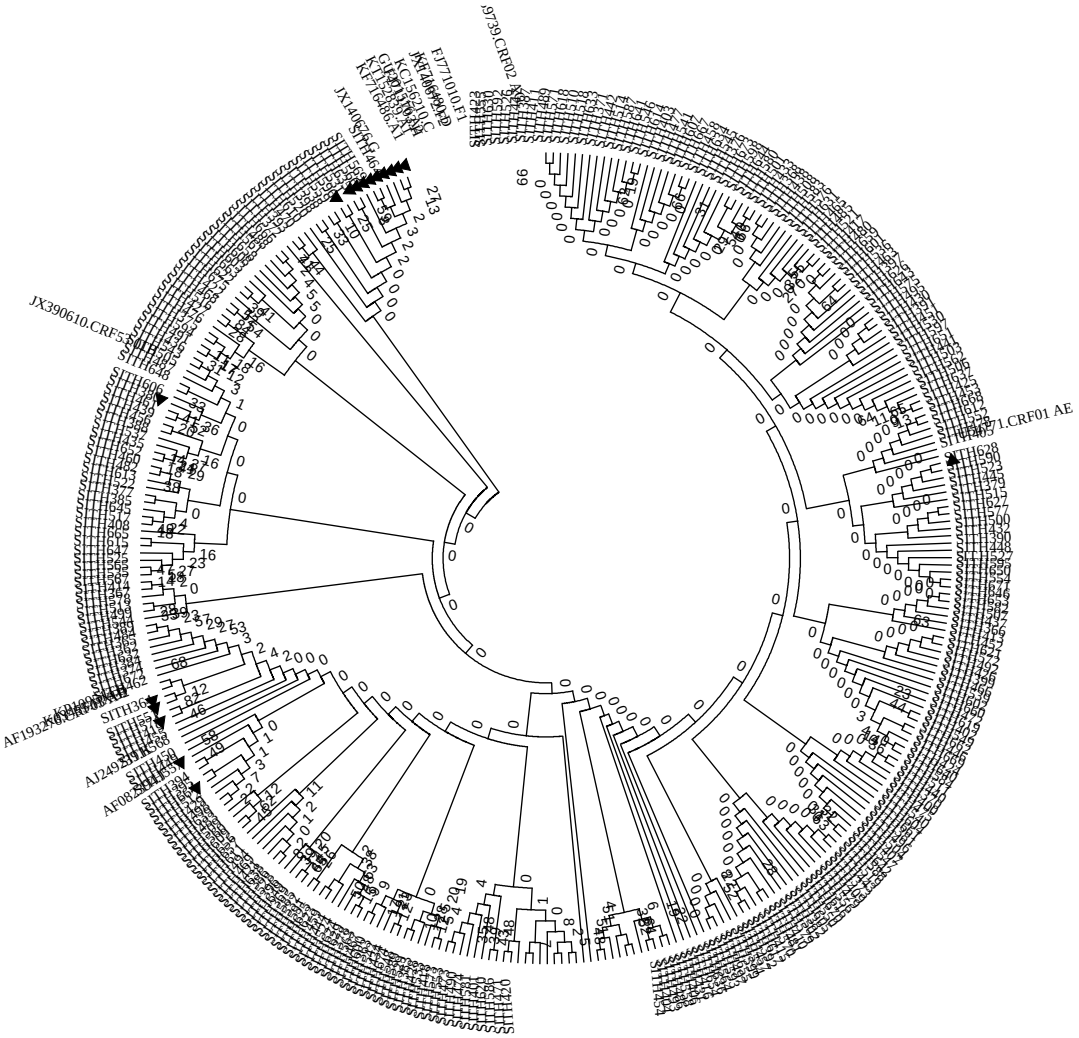
(b)

Figure 5.3 Neighbor-joining tree analysis of (a) RT nucleotide sequences and (b) PR nucleotide sequences of 250 samples collected in 2010. Labels filled triangle show HIV-1 subtype reference sequences (cont.).



(a)

Figure 5.4 Neighbor-joining tree analysis of (a) RT nucleotide sequences and (b) PR nucleotide sequences of 250 samples collected in 2010. Labels filled triangle show HIV-1 subtype reference sequences.



(b)

Figure 5.4 Neighbor-joining tree analysis of (a) RT nucleotide sequences and (b) PR nucleotide sequences of 314 samples collected in 2011. Labels filled triangle show HIV-1 subtype reference sequences (cont.).

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH001	2009	B	B	B	B
SITH002	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH003	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH004	2009	B	CRF01_AE	B	CRF01_AE
SITH005	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH006	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH007	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH008	2009	CRF01_AE	CRF01_AE	K	CRF01_AE
SITH009	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH010	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH011	2009	CRF01_AE	CRF01_AE	CRF02_AG	CRF01_AE
SITH012	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH013	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH014	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH015	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH016	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH017	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH018	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH019	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH020	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH021	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH022	2009	B	B	B	B
SITH023	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH024	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH025	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH026	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH027	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH028	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH029	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH030	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH031	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH032	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH033	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH034	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH035	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH036	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH037	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH038	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH039	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH040	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH041	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH042	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH043	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH044	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH045	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH046	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH047	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH048	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH049	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH050	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH051	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH052	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH053	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH054	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH055	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH056	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH057	2009	CRF01_AE	B	CRF01_AE	B
SITH058	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH059	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH060	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH061	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH062	2009	CRF01_AE	CRF01_AE	CRF01_AE	A2
SITH063	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH064	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH065	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH066	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH067	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH068	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH069	2009	CRF01_AE	CRF01_AE	A2	CRF01_AE
SITH070	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH071	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH072	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH073	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH074	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH075	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH076	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH077	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH078	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH079	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH080	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH081	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH082	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH083	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH084	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH085	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH086	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH087	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH088	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH089	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH090	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH091	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH092	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH093	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH094	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH095	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH096	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH097	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH098	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH099	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH100	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH101	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH102	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH103	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH104	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH105	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH106	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH107	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH108	2009	B	B	B	B
SITH109	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH110	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH111	2009	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH112	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH113	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH114	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH115	2010	B	B	B	B
SITH116	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH117	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH118	2010	B	B	B	B
SITH119	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH120	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH121	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH122	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH123	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH124	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH125	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH126	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH127	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH128	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH129	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH130	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH131	2010	B	B	B	B
SITH132	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH133	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH134	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH135	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH136	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH137	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH138	2010	B	B	B	B
SITH139	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH140	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH141	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH142	2010	CRF01_AE	CRF01_AE	CRF01_AE	A2
SITH143	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH144	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH145	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH146	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH147	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH148	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH149	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH150	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH151	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH152	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH153	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH154	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH155	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH156	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH157	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH158	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH159	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH160	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH161	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH162	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH163	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH164	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH165	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH166	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH167	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH168	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH169	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH170	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH171	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH172	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH173	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH174	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH175	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH176	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH177	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH178	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH179	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH180	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH181	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH182	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH183	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH184	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH185	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH186	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH187	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH188	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH189	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH190	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH191	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH192	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH193	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH194	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH195	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH196	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH197	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH198	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH199	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH200	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH201	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH202	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH203	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH204	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH205	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH206	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH207	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH208	2010	D	CRF01_AE	D	CRF01_AE
SITH209	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH210	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH211	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH212	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH213	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH214	2010	B	B	J	B
SITH215	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH216	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH217	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH218	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH219	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH220	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH221	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH222	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH223	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH224	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH225	2010	B	B	B	B
SITH226	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH227	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH228	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH229	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH230	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH231	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH232	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH233	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH234	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH235	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH236	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH237	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH238	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH239	2010	B	CRF01_AE	B	CRF01_AE
SITH240	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH241	2010	D	CRF01_AE	D	CRF01_AE
SITH242	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH243	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH244	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH245	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH246	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH247	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH248	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH249	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH250	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH251	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH252	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH253	2010	B	CRF01_AE	B	CRF01_AE
SITH254	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH255	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH256	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH257	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH258	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH259	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH260	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH261	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH262	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH263	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH264	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH265	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH266	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH267	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH268	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH269	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH270	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH271	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH272	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH273	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH274	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH275	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH276	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH277	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH278	2010	CRF02_AG	CRF01_AE	CRF02_AG	CRF01_AE
SITH279	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH280	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH281	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH282	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH283	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH284	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH285	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH286	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH287	2010	B	B	B	B
SITH288	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH289	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH290	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH291	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH292	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH293	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH294	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH295	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH296	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH297	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH298	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH299	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH300	2010	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH301	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH302	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH303	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH304	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH305	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH306	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH307	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH308	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH309	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH310	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH311	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH312	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH313	2010	K	CRF01AE	K	CRF01AE
SITH314	2010	B	B	B	B
SITH315	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH316	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH317	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH318	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH319	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH320	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH321	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH322	2010	B	B	B	B
SITH323	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH324	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH325	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH326	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH327	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH328	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH329	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH330	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH331	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH332	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH333	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH334	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH335	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH336	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH337	2010	B	B	B	B
SITH338	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH339	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH340	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH341	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH342	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH343	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH344	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH345	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH346	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH347	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH348	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH349	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH350	2010	B	B	B	B
SITH351	2010	CRF01AE	K	CRF01AE	K
SITH352	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH353	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH354	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH355	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH356	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH357	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH358	2010	K	CRF01AE	K	CRF01AE
SITH359	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH360	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH361	2010	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH362	2011	B	B	B	B
SITH363	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH364	2011	B	B	B	CRF03_AB
SITH365	2011	B	B	B	B
SITH366	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH367	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH368	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH369	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH370	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH371	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH372	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH373	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH374	2011	B	B	B	B
SITH375	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH376	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH377	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH378	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH379	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH380	2011	CRF01AE	CRF01AE	CRF01AE	CRF01AE
SITH381	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH382	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH383	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH384	2011	B	B	B	B
SITH385	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH386	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH387	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH388	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH389	2011	B	B	B	B
SITH390	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH391	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH392	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH393	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH394	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH395	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH396	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH397	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH398	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH399	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH400	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH401	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH402	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH403	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH404	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH405	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH406	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH407	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH408	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH409	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH410	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH411	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH412	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH413	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH414	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH415	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH416	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH417	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH418	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH419	2011	B	CRF01_AE	B	CRF01_AE
SITH420	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH421	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH422	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH423	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH424	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH425	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH426	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH427	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH428	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH429	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH430	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH431	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH432	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH433	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH434	2011	K	CRF01_AE	K	CRF01_AE
SITH435	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH436	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH437	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH438	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH439	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH440	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH441	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH442	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH443	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH444	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH445	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH446	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH447	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH448	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH449	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH450	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH451	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH452	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH453	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH454	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH455	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH456	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH457	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH458	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH459	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH460	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH461	2011	B	CRF01_AE	B	CRF01_AE
SITH462	2011	B	B	B	B
SITH463	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH464	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH465	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH466	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH467	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH468	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH469	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH470	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH471	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH472	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH473	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH474	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH475	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH476	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH477	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH478	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH479	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH480	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH481	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH482	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH483	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH484	2011	B	B	B	B
SITH485	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH486	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH487	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH488	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH489	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH490	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH491	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH492	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH493	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH494	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH495	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH496	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH497	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH498	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH499	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH500	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH501	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH502	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH503	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH504	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH505	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH506	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH507	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH508	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH509	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH510	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH511	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH512	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH513	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH514	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH515	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH516	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH517	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
		PR	RT	PR	RT
SITH518	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH519	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH520	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH521	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH522	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH523	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH524	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH525	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH526	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH527	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH528	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH529	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH530	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH531	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH532	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH533	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH534	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH535	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH536	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH537	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH538	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH539	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH540	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH541	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH542	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH543	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH544	2011	B	B	B	B
SITH545	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH546	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH547	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH548	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH549	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH550	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH551	2011	B	B	B	B
SITH552	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH553	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH554	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
SITH555	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH556	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH557	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH558	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH559	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH560	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH561	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH562	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH563	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH564	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH565	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH566	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH567	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH568	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH569	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH570	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH571	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH572	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH573	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH574	2011	D	B	D	B
SITH575	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH576	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH577	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH578	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH579	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH580	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH581	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH582	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH583	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH584	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH585	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH586	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH587	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH588	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH589	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH590	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH591	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH592	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
SITH593	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH594	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH595	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH596	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH597	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH598	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH599	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH600	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH601	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH602	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH603	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH604	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH605	2011	CRF01_AE	B	CRF01_AE	B
SITH606	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH607	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH608	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH609	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH610	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH611	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH612	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH613	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH614	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH615	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH616	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH617	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH618	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH619	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH620	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH621	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH622	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH623	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH624	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH625	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH626	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH627	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH628	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH629	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH630	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
SITH631	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH632	2011	B	B	B	B
SITH633	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH634	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH635	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH636	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH637	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH638	2011	K	CRF01_AE	K	CRF01_AE
SITH639	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH640	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH641	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH642	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH643	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH644	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH645	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH646	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH647	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH648	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH649	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH650	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH651	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH652	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH653	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH654	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH655	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH656	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH657	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH658	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH659	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH660	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH661	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH662	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH663	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH664	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH665	2011	CRF01_AE	CRF01_AE	A2	CRF01_AE
SITH666	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH667	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH668	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

Table 5.2 HIV-1 subtypes determined by Stanford HIV database and phylogenic tree analysis of RT and PR nucleotide sequences (cont.)

Sample ID	Years	HIV subtype			
		Stanford analysis		Phylogenetic tree analysis	
SITH669	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH670	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH671	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH672	2011	B	B	B	B
SITH673	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH674	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE
SITH675	2011	CRF01_AE	CRF01_AE	CRF01_AE	CRF01_AE

5.3 HIV drug resistant analysis

HIV-1 *pol* nucleotide sequences were analyzed by examining amino acid mutations at the PR and RT sites associated with antiretroviral drug resistance by using Viroseq software version 2.8 (Celera, USA) and HIVdb program in the HIV Drug Resistance Database from Stanford University (http://hivdb.stanford.edu/).

5.3.1 Prevalence of HIV-1 drug resistance mutation codon of *pol* gene

A total of. HIV-1 drug resistance mutation codons of 111, 250 and 354 samples obtained from year 2009, 2010 and 2011 were analyzed, respectively.

Antiretroviral drugs, RT–inhibitors, related resistance codons was characterized as NRTIs and NNRTIs. HIV drugs resistance associated mutations (DRMs) against NRTIs observed were K65R, K70R/E, L74V, Y115F, M184V, M41L, D67G, L210M, T215A, K219Q, E44A, A62V, T69N and Q1581R. Among these mutations, M184V was the most prevalent DRMs against NRTIs. DRMs to NNRTIs observed were L100I, K101E, K103N, V106A, E138A, V179F, Y181C, Y188L, G190A, F227L and M230L. Among these mutations, Y181C was the most prevalent mutation associated with resistance to NNRTIs. DRMs to Protease inhibitors (PIs)observed were V32I, M46I/L, M47I/V/A, G48V/M, I50L/V, I54F/I/L/V/A, L76V, V82A/F/M, I84V, N88S and L90M. Among these mutations, V82A/F/M was found predominantly. The most frequency of secondary mutation was found at L89I/V/MV/IMV.

In 2009 samples, the frequency of DRMs to NRTIs were M184V/MV/IMV/IM/I (65.5%, 73/111), K70KR/S/T/KN/EG/G/R/W/KQ/Q/E (31.9%, 35/111), T69N/I/DN/G/NT/A/AT/K/AGST/ST/S/D (17.4%, 19/111), T215/A/C/Y/D/ST/AT/NY/FY/V/I/IT/CY/F (23.4%, 26/111). The frequency of DRMs to NNRTIs were Y181C/CY/G/FY/I/V/HY/L (37.8%,42/111), K101E/EKQ/HKNQ/DEHQ/KQPT/AE/A/EK/Q/KQ/DH/H/HN/KN/PQ/ILKQ/P/HPQ (20.7%, 23/111), G190A/AG/S/C (22.4%, 25/111). DRMs to PIs were characterized as major PIs drug resistance and minor PIs drug resistance mutations. The frequency of major PIs drug resistance codon mutations were V82/A/F/M/IM (10.5%, 17/111), V32I(0.5%, 1/111), M46I/L/IM/LM(1%, 1/111), I47A/V(1%, 1/111), I54F/I/L/V/A(1%,1/111), L90M/LM(1.4%,2/111). The frequency of minor PI drug resistance codon mutations were L63P(21.8%, 24/111).

In 2010 samples, the frequency of DRMs to NRTIs were M184V/MV/IMV/IM/I(49.31%,123/250),K70KR/S/T/KN/EG/G/R/W/KQ/Q/E (12.62%,32/250), T69N/I/DN/G/NT/A/AT/K/AGST/ST/S/D (17.4%,44/250), T215/A/C/Y/D/ST/AT/NY/FY/V/I/IT/CY/F (14.21%,36/250). The frequency of DRMs to NNRTIs were Y181C/CY/G/FY/I/V/HY/L (34.31%, 86/250), K101E/EKQ/HKNQ/DEHQ/KQPT/AE/A/EK/Q/KQ/DH/H/HN/KN/PQ/ILKQ/P/HPQ (16.6%, 42/250), G190A/AG/S/C (18.21%, 46/250). The frequency of major PIs drug resistance codon mutations wereV82/A/F/M/IM (1.2%,3/250), M46I/L/IM/LM(1.6%, 4/250), I47A/V(0.2%, 1/250), I54F/I/L/V/A(1%,3/250) and L90M/LM(1%,3/250). The frequency of minor PI drug resistance codon mutations were L63P(19%, 48250).

In 2011 samples, the frequency of DRMs to NRTIs were M184V/MV/IMV/IM/I(58.32%,183/314),K70KR/S/T/KN/EG/G/R/W/KQ/Q/E(13.84 %,43/314), T69N/I/DN/G/NT/A/AT/K/AGST/ST/S/D (14.19%, 45/314), T215/A/C/Y/D/ST/AT/NY/FY/V/I/IT/CY/F (16.99%,53/314). The frequency of DRMs to NNRTIs were Y181C/CY/G/FY/I/V/HY/L (33.63%,106/314), K101E/EKQ/HKNQ/DEHQ/KQPT/AE/A/EK/Q/KQ/DH/H/HN/KN/PQ/ILKQ/P/HPQ (15.59%, 49/314), G190A/AG/S/C (22.4%, 70/314). The frequency of major PI s drug resistance codon mutations were V82/A/F/M/IM (2.28%,7/314), M46I/L/IM/LM(2.63%, 8/314), I47A/V(0.18%,1/314) I50L/V(0.35%, 1/314)),

I54F/I/L/V/A(1.93%,6/314), L76V(1.4%,4/314) and L90M/LM(0.53%,2/314). The frequency of minor PI drug resistance codon mutations were L63P(0%, 0/314).

Frequency of DRMs to PIs, NRTIs and NNRTIs were shown in tables 5.3-5.6

Table 5.3 Percentage of DRMs prevalence against antiretroviral drugs.

DRMs to	**2009**	**2010**	**2011**
PIs	7.9	6.8	8.9
NRTIs	66.1	55.2	63.7
NNRTIs	81.2	64.8	70.7
Single PI	0.6	3.2	1.9
Single NRTI	1.8	3.2	3.5
Single NNRTI	7.9	12.4	9.9
NRTIs+NNRTIs	61.3	49.2	54.5
PIs+NRTIs+NNRTIs	4.8	2.8	5.1

Table 5.4 Percentage of DRMs prevalence to Protease Inhibitors

DRMs to PIs	**2009**	**2010**	**2011**
V32I	0.5	0	0
M46I/L/IM/LM	1	1.6	2.63
I47A/V	1	0.2	0.18
G48V/M	0	0	0
I50L/V	0	0	0.35
I54F/I/L/V/A	1	1	1.93
L76V	0	0	1.4
V82/A/F/M/IM	10.5	1.2	2.28
I84V/IV	0.9	0.2	0.70
N88S	0	0.6	0
L90M/LM	1.4	1	0.53
L10I/IV/IVL/IL/IM/F/FL/FY/V/LV	30.5	31.6	33.63
V11I	0	0.4	0.18
I13V	0	0	0
L33I/F	2.7	2.4	3.68
I47IL	1	0.2	0
F53L/Y	0.5	0.2	0
K43T	5	0	0
Q58E/EQ	0.9	0.2	0
L63P	21.8	19	0
A71T/AV/V/IMV	1.4	3	2.63
T74S/ST	0.5	0.4	0.35
N83D/DN/NDT	0	0.6	0.18
I85V/IV	0	0.2	0
N88H/N/D/S	0.5	0.2	0
L89I/V/MV/IMV	89	0.6	1.4

Table 5.5 Percentage of DRMs prevalence to NRTIs

DRMs	**2009**	**2010**	**2011**
K65R/N/KR	9.1	4.61	5.25
K70KR/S/T/KN/EG/G/R/W/KQ/Q/E	31.9	12.62	13.84
L74V/IL/I/LV	6.4	4.2	5.6
Y115F/FY	1.8	1.2	1.05
M184V/MV/IMV/IM/I	65.5	49.31	58.32
M41L/MT/LM	8.2	7.81	9.81
D67G/N/S/E/DG/DN/T	22.5	20.62	19.79
L210M/W/F/LW/T/S	12.8	7.41	6.3
T215/A/C/Y/D/ST/AT/NY/FY/V/I/IT/CY/F	23.4	14.21	16.99
K219Q/EGQR/KT/KR/KQ/D/H/KN/R/N/HQ/EK/E	19.2	15.61	14.54
E44A/AE/ED/D	4.6	2.2	0.18
A62AV/V	4.6	3.41	1.93
T69N/I/DN/G/NT/A/AT/K/AGST/ST/S/D	17.4	13.62	14.19
Q151QR/KM/M	5	4.41	3.68

Table 5.6 Percentage of DRMs prevalence to NNRTIs

DRMs	**2009**	**2010**	**2011**
L100I/F	1.4	1.4	0.88
K101E/EKQ/HKNQ/DEHQ/KQPT/	20.7	16.6	15.59
K103N/A/T/S/KN/NS/KR/R	31	21.2	25.57
V106A/I/M/IMV/IV	10.1	6.6	4.73
E138/A/EG/EK/AG/Q/G/K/S	7	2.6	1.58
V179F/T/D/DE/DN/DV/E/S	6.8	6.21	8.06
Y181C/CY/G/FY/I/V/HY/L	37.8	34.31	33.63
Y188L/C/W/FL/HY/CY/FHLY	3.7	1.8	6.3
G190A/AG/S/C	22.4	18.21	22.24
F227L/FL	1.9	2	1.75
M230L/LM	3.7	4	2.28

5.4 HIV-1 nearly full-length genomic sequencing analysis.

A total of 35 samples from 675 samples were randomly selected for HIV-1 nearly full-length genomic sequencing analysis.The demographic data of these 35 samples was shown in Table 4.3. The average age of 35 samples studiedsamples was 30.62 years. The samples of this study included 54.29% (male) and 45.71% (female). The average of HIV viral load and $CD4^+$cells was log 4.68copies/ml and 266.91 cell/mm^3, respectively.

Table 5.7 Demographic, genotype and ART resistance mutation characteristics of 35 studied samples.

Sample ID	Gender	Age (y)	Year of diagnosis	Location	$CD4^+$ count (cells/ mm^3)	Viral load[a] (copies/ml)	ART status[b]	HIV subtypes		ART resistant mutation
								NFLG	*pol*	
SITH438	M	18	2011	Central	353	4,545	AZT,ddI, LPV/RTV	01_AE	01_AE	D67N,V179D, Y181FINY
SITH496	F	25	2011	Northern	47	45,123	d4T,3TC, EFV	01_AE	01_AE	V179D
SITH467	F	41	2011	Northern	698	54,523	AZT,3TC, NVP	01_AE	01_AE	D67N, K70KR,A98G, K101E, G190S
SITH458	F	17	2011	Northern	235	2,231	d4T,3TC, LPV/RTV	01_AE	01_AE	M184V,L90M
SITH436	F	19	2011	Northern	365	542,165	LPV/RTV ,TDF,AZT,3TC	01_AE	01_AE	D67N, T69D, T215AITV, K219Q,K101E, Y181C
SITH587	F	49	2011	Northeastern	431	4,000	AZT,ddI, LPV/RTV	01_AE	01_AE	-
SITH651	F	36	2011	Northeastern	52	2,223	d4T,3TC, EFV	01_AE	01_AE	M184V,K103N

Table 5.7 Demographic, genotype and ART resistance mutation characteristics of 35 studied samples (cont.)

Sample ID	Gender	Age (y)	Year of diagnosis	Location	$CD4^+$ count (cells/ mm^3	Viral load[a] (copies/ml)	ART status[b]	HIV genotypes		ART resistant mutation
								NFLG	*pol*	
SITH669	F	36	2011	Northeastern	632	21,213	d4T,3TC ,NVP	01_AE	01_AE	M184IV,Y181 C
SITH632	M	15	2011	Northeastern	547	56,321	ddI,3TC, LPV/RT V	01_AE	B	M184V,Y188L
SITH609	F	18	2011	Northeastern	285	142,489	AZT,3T C,LPV/R TV	01_AE	01_AE	-
SITH663	M	34	2011	Northeastern	524	7,278	d4T,3TC ,EFV	01_AE	01_AE	M184V, T215F,K103RS , V106IV
SITH613	F	23	2011	Northeastern	147	10,077	AZT,LP V/RTV, TDF	01_AE	01_AE	L210LS,V179 D, Y188L
SITH617	M	38	2011	Northeastern	896	5,663	d4T,3TC ,NVP	01_AE	01_AE	M184V,K103N ,L10I
SITH647	M	16	2011	Northeastern	146	166,628	d4T,3TC ,NVP	01_AE	01_AE	M184V,K103N , Y181C,L10IV

Table 5.7 Demographic, genotype and ART resistance mutation characteristics of 35 studied samples (cont.)

Sample ID	Gender	Age (y)	Year of diagnosis	Location	$CD4^+$ count (cells/ mm^3	Viral load[a] (copies/ml)	ART status[b]	HIV genotypes		ART resistant mutation
								NFLG	pol	
SITH640	F	46	2011	Northeastern	285	22,229	TDF,EFV, 3TC	01_AE	01_AE	M184V, K219EK,L10I
SITH643	M	24	2011	Northeastern	125	77,789	d4T,3TC,N VP	01_AE	01_AE	-
SITH629	F	33	2011	Northeastern	541	23,214	d4T,3TC,N VP	01_AE	01_AE	V75LV,K103KN
SITH572	F	48	2011	Southern	4	58,562	d4T,3TC,N VP	01_AE	01_AE	-
SITH582	F	35	2011	Southern	741	4,976	AZT,3TC, EFV	01_AE	01_AE	-
SITH299	F	15	2010	Northern	245	8,745	d4T,3TC,L PV/RTV	01_AE	01_AE	D67G,K70E,k103s, V106A
SITH225	M	14	2010	Northern	344	4,563	AZT,3TC,I DV,RTV	01_AE	B	T69N,V75I,K103N, Y181C

Table 5.7 Demographic, genotype and ART resistance mutation characteristics of 35 studied samples (cont.)

Sample ID	Gender	Age (y)	Year of diagnosis	Location	$CD4^+$ count (cells/ mm^3	Viral load[a] (copies/ml)	ART status[b]	HIV genotypes		ART resistant mutation
								NFLG	pol	
SITH195	M	37	2010	Northern	745	3,194	d4T,3TC ,NVP	01_AE	01_AE	-
SITH282	F	50	2010	Northern	236	41,236	d4T,3TC ,NVP	01_AE	01_AE	M184V,D67N,Y181 C
SITH316	F	21	2010	Northern	321	6,325	d4T,3TC ,NVP	01_AE	01_AE	-
SITH349	F	46	2010	Southern	124	86,249	d4T,3TC ,NVP	01_AE	01_AE	-
SITH322	M	21	2010	Southern	4	341,861	d4T,3TC ,NVP	01_AE	B	,V75A,M184V,Y181I
SITH079	F	12	2009	Southern	490	2,000	AZT,3T C,IDV,L PV/RTV	01_AE	01_AE	M184V,L210W,M46I ,I54V
SITH085	M	39	2009	Southern	8	8,186	AZT,3T C,NVP	01_AE	01_AE	M184V,K219E

Table 5.7 Demographic, genotype and ART resistance mutation characteristics of 35 studied samples (cont.)

Sample ID	Gender	Age (y)	Year of diagnosis	Location	CD4$^+$ count (cells/ mm^3	Viralload[a] (log copies/ml)	ART status[b]	HIV genotypes		ART resistant mutation
								NFLG	*pol*	
SITH103	F	31	2009	Southern	210	35,421	d4T,3TC, EFV	01_AE	01_AE	L210F,L10V
SITH106	F	48	2009	Southern	960	3,194	GPO-VIRS	01_AE	01_AE	M184IK101ILKQ,Y181C,G190A
SITH610	M	44	2011	Northeastern	231	25,882	NONE	01_AE	01_AE	-
SITH642	M	37	2011	Northeastern	24	57,427	NONE	01_AE	01_AE	-
SITH627	M	44	2011	Northeastern	415	1,211	NONE	01_AE	01_AE	-
SITH184	F	17	2010	Northern	228	11,629	NONE	01_AE	01_AE	-
SITH110	F	25	2009	Southern	361	8,105	NONE	01_AE	01_AE	-

Abbreviations: F, female; M, male; ND, not done; NFLG, nearly full-length genome.

[a] HIV viral load was determined by Abbot m2000rt quantitative assay of all samples collected during January 2009- December 2011[b]. All subjects were received ART starting on NRTI, NNRTI, major protease inhibitor resistant mutation [c] minor protease inhibitor resistant mutation

HIV-1 subtypes were determined by phylogenetic analysis of 35 nearly full-length genomic sequences. HIV-1 nucleotide sequences covered *gag*, *pol* and *env* (8,764 bp.) regions were assembled using DNA Baser and aligned sequences by MEGA 7 analysis. Phylogenetic tree analysis and statistical robustness of the Maximum Likelihood methods were based on the Tamura-Nei model.

Sequences of reference strains belonging to different subtypes were showed in table 5.8

Table 5.8 HIV-1 subtype reference sequences used for phylogenetic tree analysis

Reference subtype	Accession number	Country
01_AE	U54771, KP109513	Thailand
	KP718930	Cameroon
	KF859741	Japan
	KP411841	Sweden
A1	KT152839	India
	KF716486	Uganda
	KP718928	Cameroon
A2	GU201516	Cameroon
	AF286238	D.R.C.
	AF286237	Cyprus
AB	AF193276	Russia
AG	KF859739	Cameroon
	EU884501	Spain
B	KJ849801	Brazil
	KJ140263	S. Korea
	K03455	France
	KP109514	Thailand
C	KC870038	China
	JX140663	Brazil
	KT022371	Kenya

Table 5.8 HIV-1 subtype reference sequences used for phylogenetic tree analysis (cont.).

Reference subtype	Accession number	Country
D	KF716480, KF716479	Uganda
	JX140670	Cameroon
	DQ054367	S. Korea
F1	FJ771010, KJ849782	Brazil
	KJ883138	Spain
F2	JX140672, AY371158, JX140673	Cameroon
G	JX140676	Cameroon
	JN106043	China
	GU362882	Spain
H	FJ711703	United Kingdom
	AF190127, AF190128	Belgium
J	AF082394, AF082395	Sweden
	EF614151	D.R.C.
K	AJ249239	Cameroon
	AJ249235	D.R.C.
CRF34_01B	EF165539, EF165540, EF165541	Thailand
CRF52_01B	DQ354113, AY945734,	Thailand
	DQ366664	Malaysia

5.4.1 Phylogenetic tree of near-full-length HIV- 1 genome (*gag, pol* and *env*).

HIV-1 nearly full-length genomic sequences from 35 samples (SITH079, SITH085, SITH103, SITH110, SITH184, SITH195, SITH225, SITH282, SITH299, SITH316, SITH322, SITH349, SITH436, SITH438, SITH458, SITH467, SITH496, SITH572, SITH582, SITH587, SITH609, SITH610, SITH613, SITH617, SITH627, SITH629, SITH632, SITH640, SITH642, SITH643, SITH647, SITH651, SITH663, SITH669) were assembled with product size 8,764 bp. covered nucleotide positions 790 - 8,795 (HXB2 numbering system) and analyzed by phylogenetic tree analysis using Maximum Likelihood method. Phylogenetic tree of these NFLG nucleotide sequences and reference sequences were shown in Figure 5.5.

Phylogenetic tree analysis showed that all 35 samples were related to reference subtype CRF01_AE.

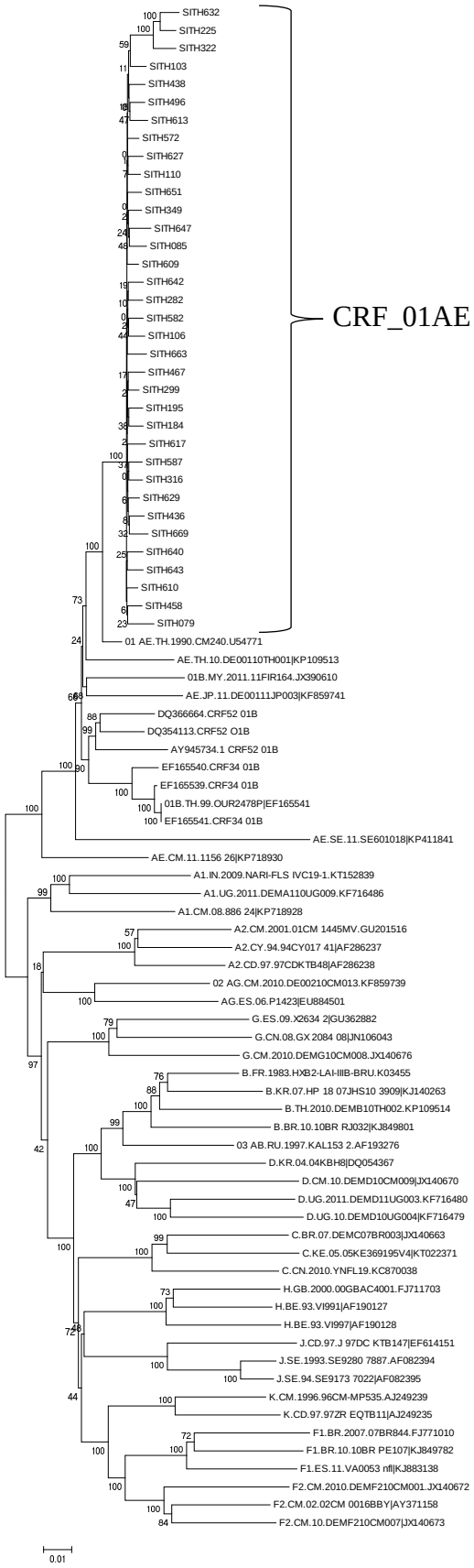


Figure 5.5 Phylogenetic tree analysis of 35 samples near-full-length HIV- 1 nucleotide sequence (8,764 bp.). Labels fill triangle show the patient's sample and unfilled triangle show HIV-1 subtype reference sequences

5.5 HIV-1 genotypic recombination analyzed by jpHMM online tool, RIP and bootscanning plot

The jumping profile Hidden Markov Model (jpHMM) which is an online subtyping and RIP (Recombinant Identification Program) recombination tool was used for recombination analysis. All HIV-1 *gag*, *pol* and *env* nucleotide sequences were analyzed by jpHMM**.** HIV-1 nucleotide sequences (*gag*, *pol* and *env* regions) from SITH632, SITH225 and SITH322 samples revealed recombination between subtypes CRF01_AE and B as shown in table 5.9. All three CRF01_AE/B recombinant strains (SITH225, SITH322 and SITH632 contained CRF01_AE backbone with the recombinant break point for subtype B in *pol* gene at nucleotide positions 2,281-3541 All 3 recombinant strains were phylogenically analyzed with other CRF01_AE/B recombinant reference sequences as show in figure 5.6. Recombination analysis of HIV-1 nearly full-length genomic sequences by RIP and bootscaning plot was shown in table 5.10. All three recombinant strains, SITH225, SITH322 and SITH632 were clustered with reference CRF34_01/B and CRF52_01/B which were CRF01/B recombinant strains from Thailand and Malaysia. SITH225, SITH322 and SITH632 samples were collected from Northern, Southern and Northeastern parts of Thailand.

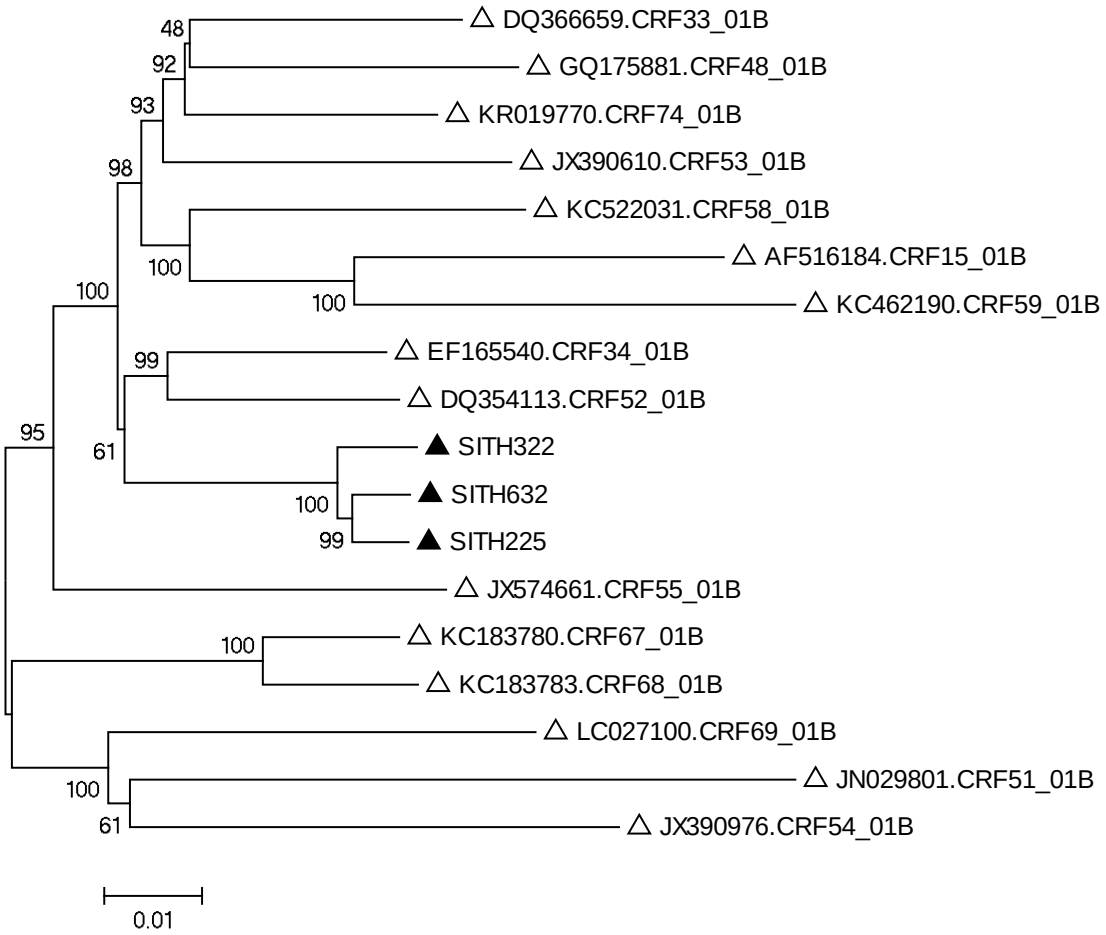


Figure 5.6 Phylogenetic tree analysis of SITH632, SITH225 and SITH322 nucleotide sequence (8,764 bp.) comparing in the CRF_01AE/B group. Labels fill triangle show the patient's sample and unfilled triangle show HIV-1 subtype reference sequence**s** including CRF15_01B, CRF33_01B, CRF34_01B, CRF48_01B, CRF51_01B, CRF55_01B,CRF58_01B, CRF59_01B, CRF67_01B, CRF68_01B,CRF74_01B and CRF76_01B

Table 5.9 Recombination analysis of HIV-1 nearly full-length genomic sequences (*gag*, *pol* and *env* genes) by jpHMM

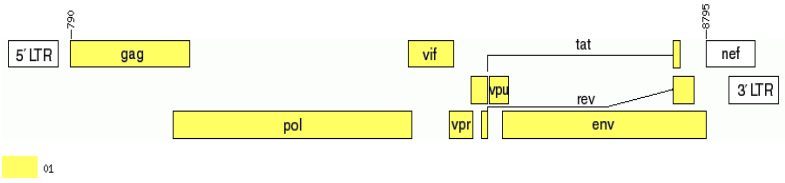
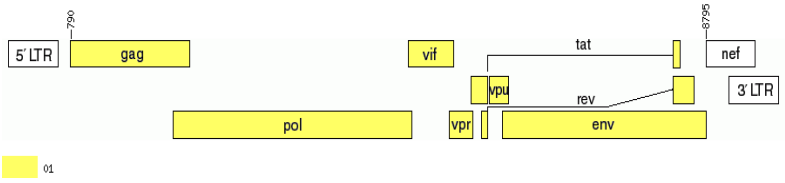
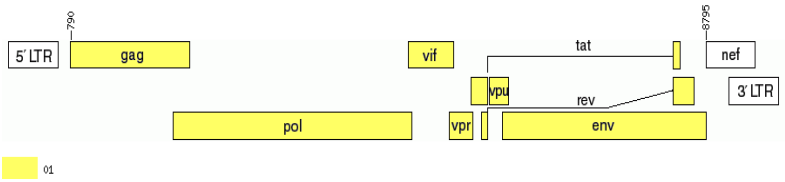
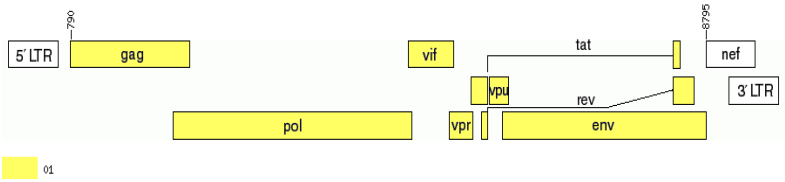
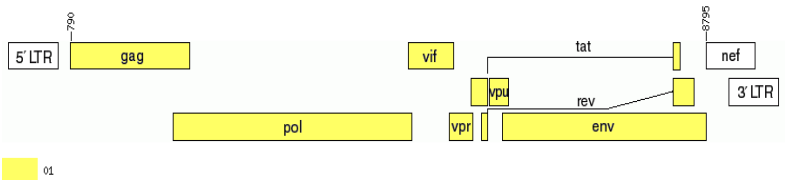
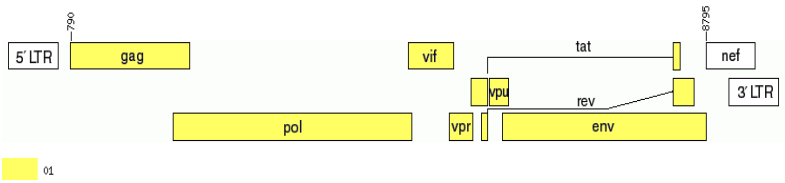
Sample	Subtype	Result
SITH438	01_AE	
SITH496	01_AE	
SITH467	01_AE	
SITH458	01_AE	
SITH436	01_AE	
SITH587	01_AE	

Table 5.9 Recombination analysis of HIV-1 nearly full-length genomic sequences (*gag*, *pol* and *env* genes) by jpHMM (cont.)

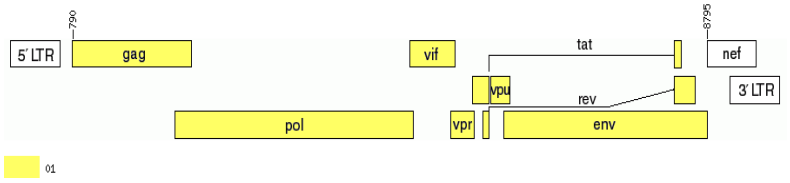
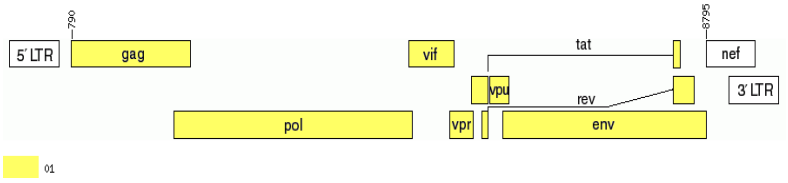
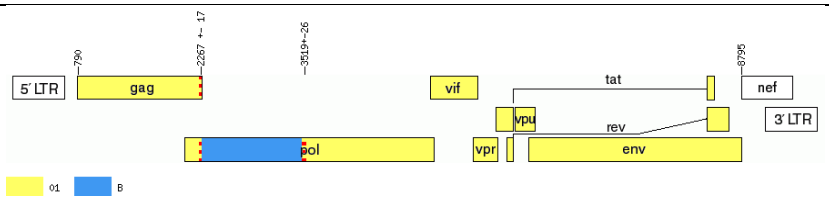
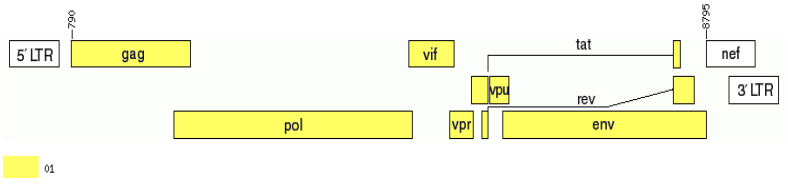
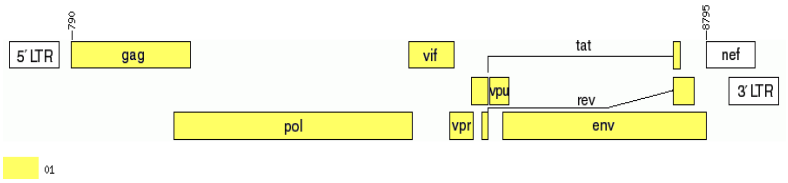
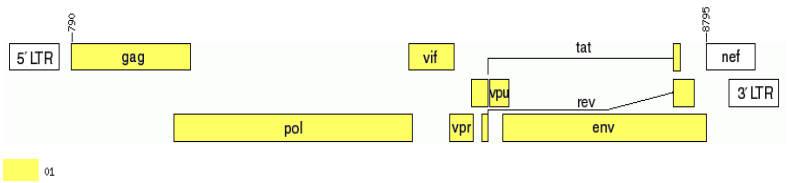
Sample	Subtype	Result
SITH651	01_AE	
SITH669	01_AE	
SITH632	01_AE, B	
SITH609	01_AE	
SITH663	01_AE	
SITH613	01_AE	

Table 5.9 Recombination analysis of HIV-1 nearly full-length genomic sequences (*gag*, *pol* and *env* genes) by jpHMM (cont.)

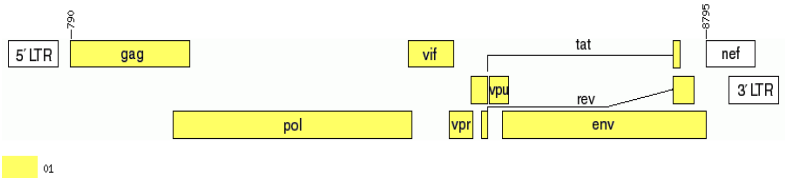
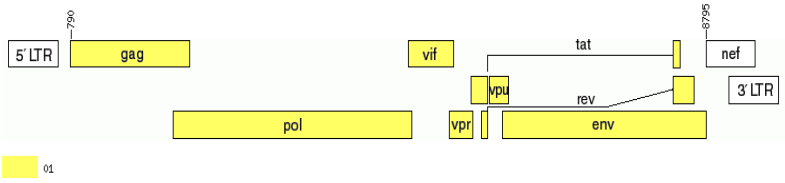
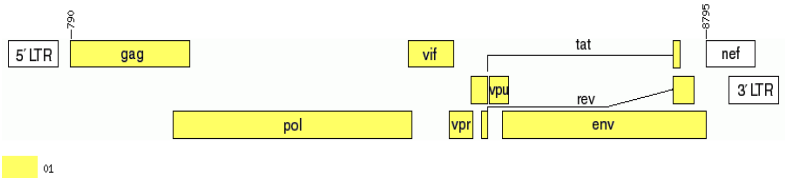
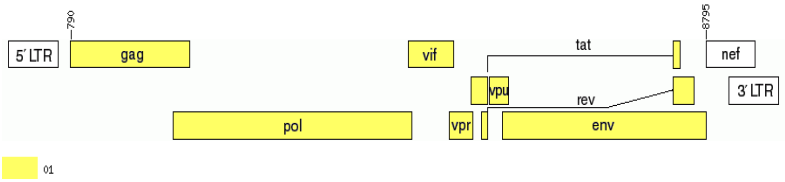
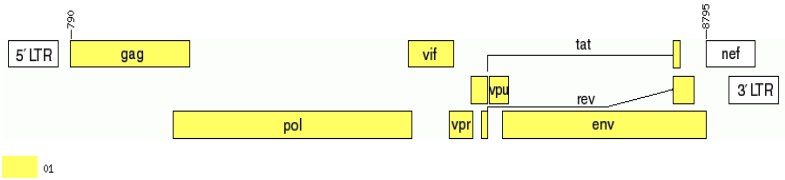
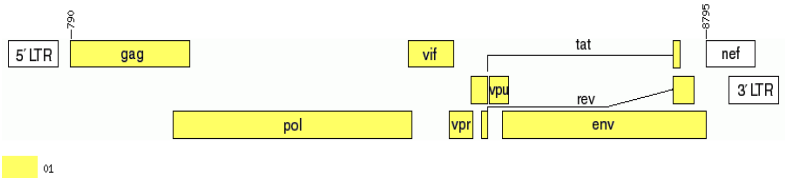
Sample	Subtype	Result
SITH610	01_AE	
SITH617	01_AE	
SITH642	01_AE	
SITH647	01_AE	
SITH627	01_AE	
SITH640	01_AE	

Table 5.9 Recombination analysis of HIV-1 nearly full-length genomic sequences (*gag*, *pol* and *env* genes) by jpHMM (cont.)

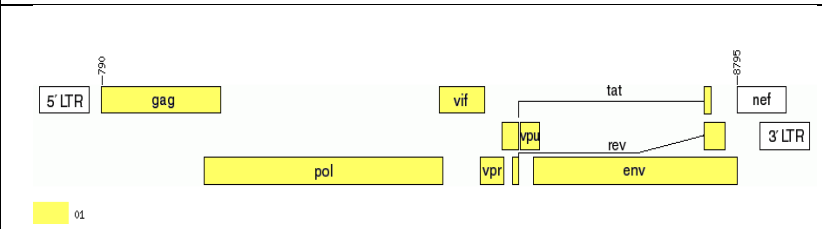
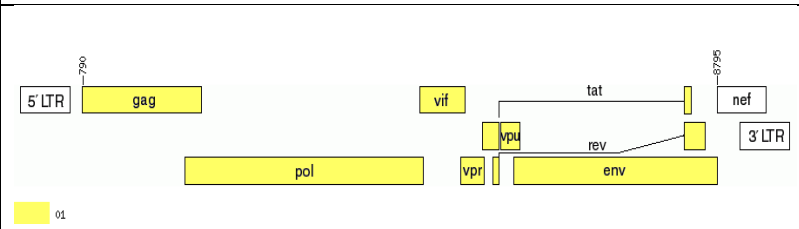
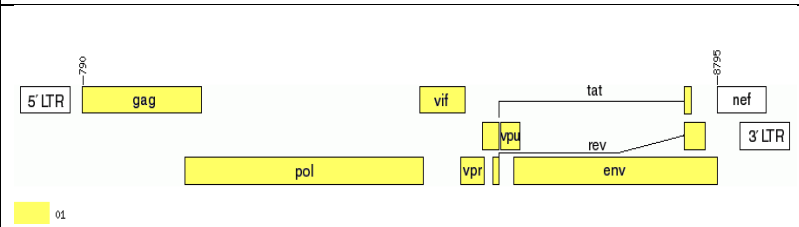
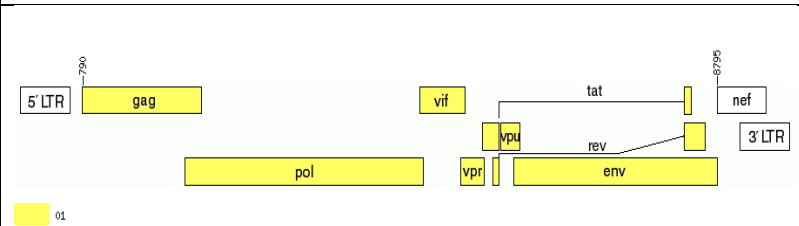
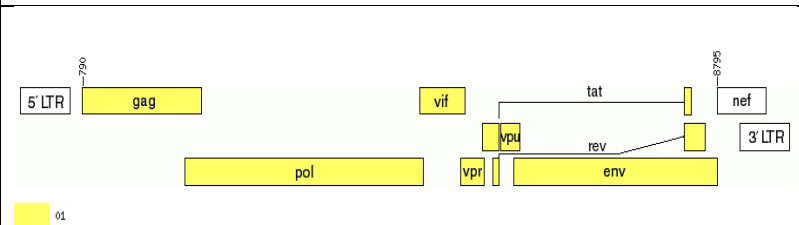
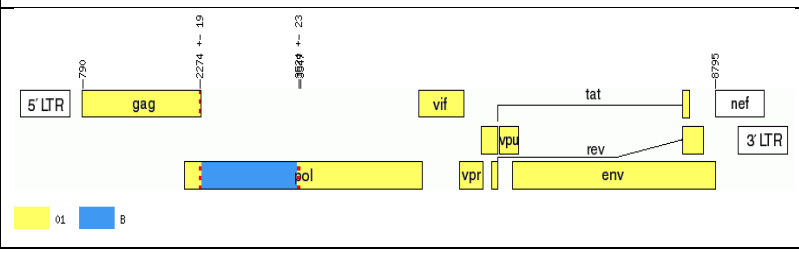
Sample	Subtype	Result
SITH643	01_AE	
SITH629	01_AE	
SITH572	01_AE	
SITH582	01_AE	
SITH299	01_AE	
SITH225	01_AE, B	

Table 5.9 Recombination analysis of HIV-1 nearly full-length genomic sequences (*gag*, *pol* and *env* genes) by jpHMM (cont.)

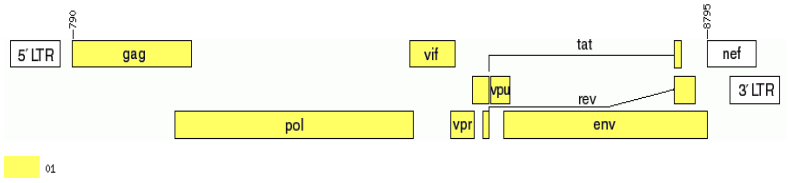
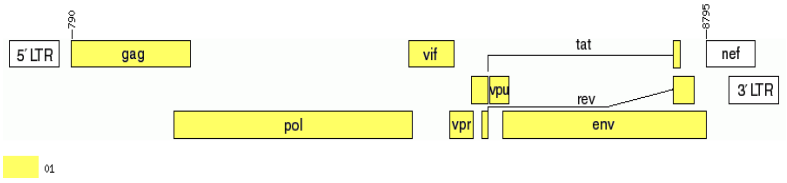
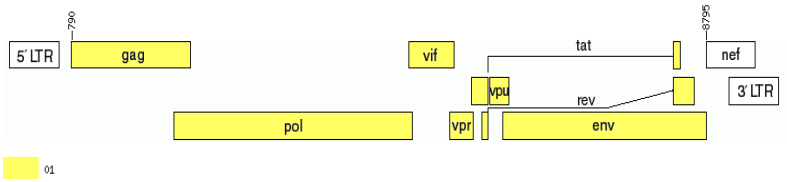
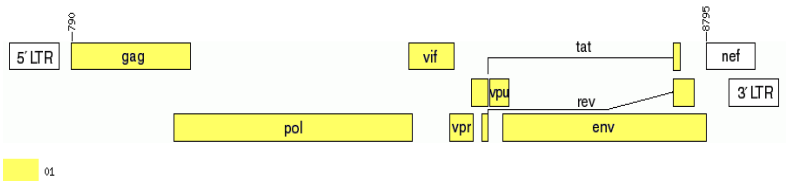
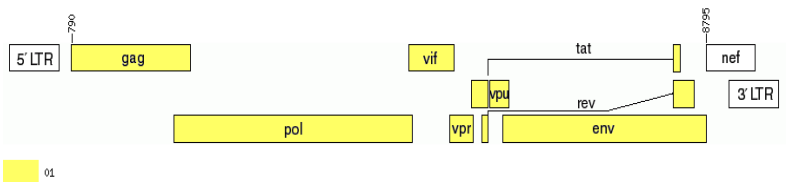
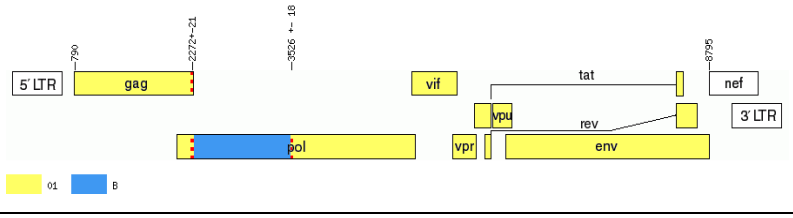
Sample	Subtype	Result
SITH195	01_AE	
SITH282	01_AE	
SITH184	01_AE	
SITH316	01_AE	
SITH349	01_AE	
SITH322	01_AE	

Table 5.9 Recombination analysis of HIV-1 nearly full-length genomic sequences (*gag*, *pol* and *env* genes) by jpHMM (cont.)

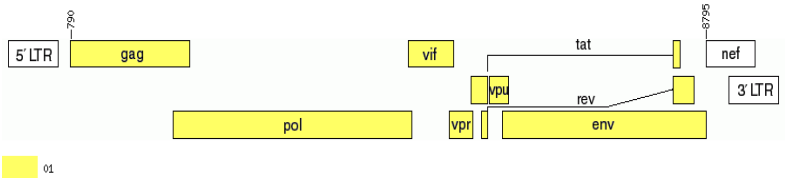
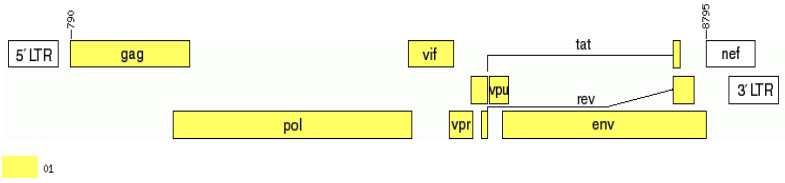
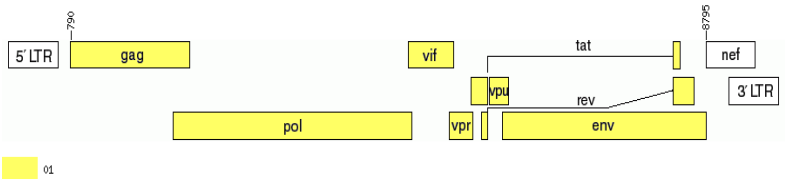
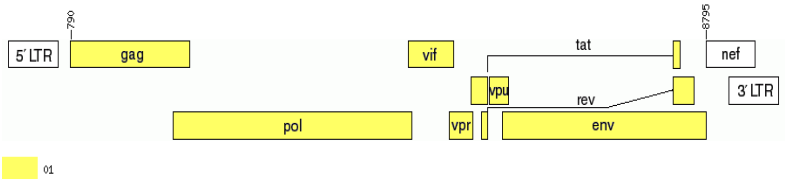
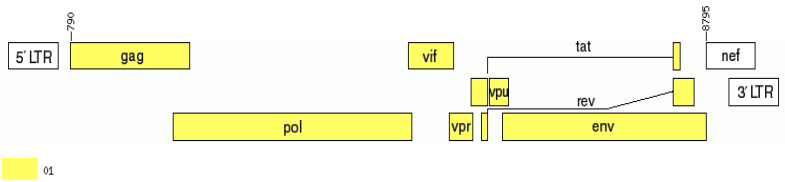
Sample	Subtype	Result
SITH079	01_AE	
SITH085	01_AE	
SITH110	01_AE	
SITH103	01_AE	
SITH106	01_AE	

Table 5.10 Recombination analysis of HIV-1 nearly full-length genomic sequences by RIP and bootscaning plot

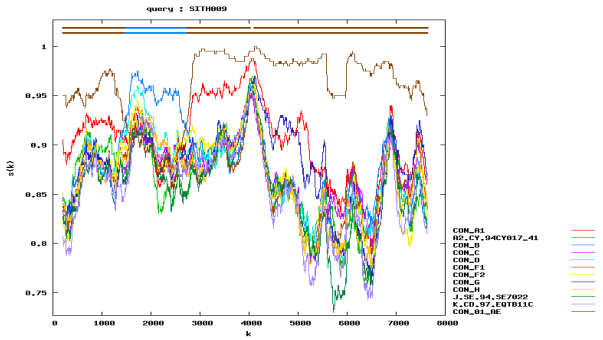
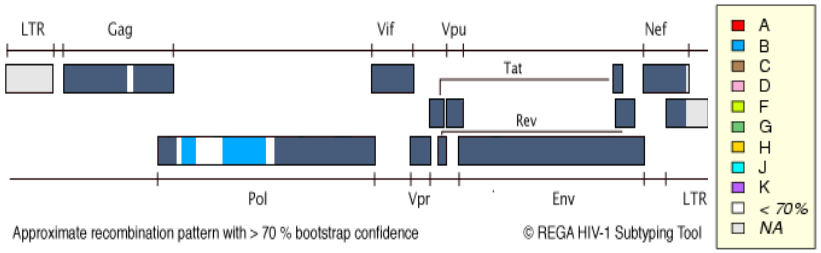
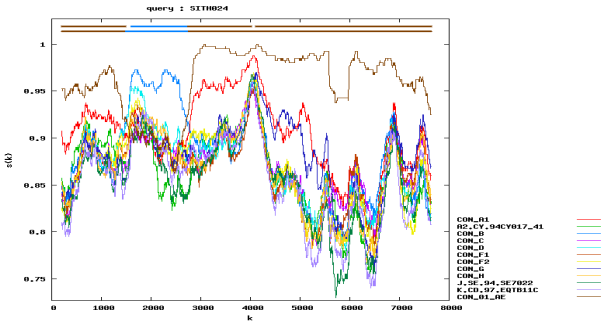
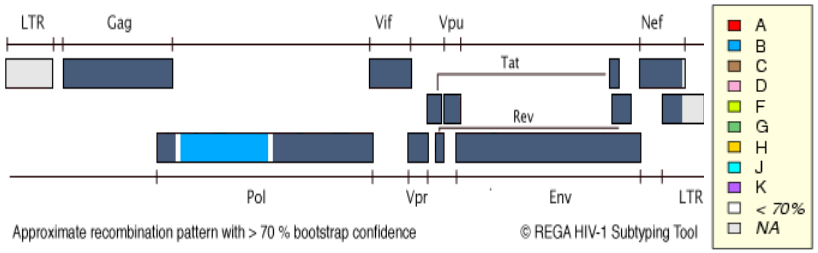
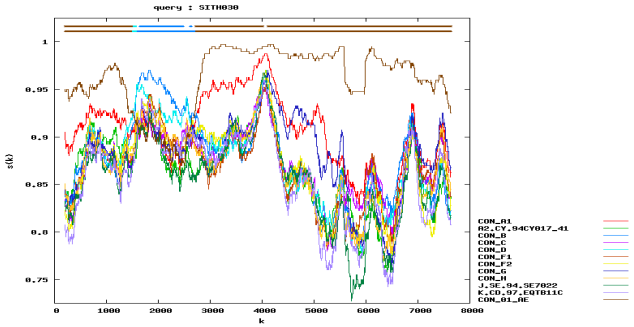
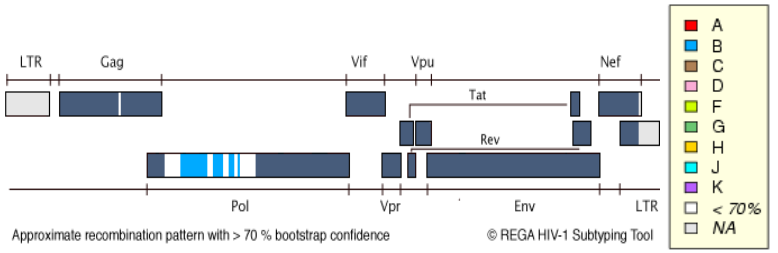
Sample ID	Bootscanning plot	Recombination map
SITH632		
SITH225		

Table 5.10 Recombination analysis of HIV-1 nearly full-length genomic sequences by RIP and bootscaning plot (cont.)

Sample ID	Bootscanning plot	Recombination map
SITH322		

CHAPTER VII
CONCLUSION

The prevalence of HIV subtype, year 2009-2011 from four area of Thailand was studied by using 675 samples sent for HIV drug resistant genotyping assays at Siriraj Hospital. CRFO1_AE (2009 = 94.6%, 2010 = 93.2%, 2011 = 93.9%) was still HIV subtype found mostly in Thailand. Moreover, HIV subtype B was secondly found. Three CRF01/B recombinant strains, clustered with CRF34_01B and CRF52_01B strains from Thailand and Malaysia were reported from HIV-1 nearly full-length genomic sequencing study of randomly 35 samples.

The most prevalence drug resistance mutations of NRTIs, NRTIs and PIs were M184V/MV/IMV/IM/I, Y181C/CY/G/FY/I/V/HY/L and L63P and M46I/L/IM/LM, respectively.

High percentages (8.6%) of CRF01/B reported here from samples collected from Southern, Northern and Northeastern provinces of Thailand might be the preliminary data to urge for intensively molecular epidemiological study of HIV genotype spread in Thailand.

CHAPTER VI
DISCUSSIONS

In Thailand, the cumulative number of HIV cases reported between 1984 - 2014 was 388,621. The newly infection cases was 7,816 in 2014 which were decreasing when comparing to 2000 (29,619 cases). The estimated number of AIDS related death decreased by 63% between 2000 and 2010 (from 55,531 to 20,670). This data indicated that after Thai government has provided free of charge of antiretroviral (ART) for people who infected with HIV which called National Access to Antiretroviral Programs for People who have AIDS or NAPHA, the number of death patients were decreasing. The coverage of antiretroviral treatment was increasing by 64.6% in 2010, 80.3% in 2013 and decreasing by 61% in 2014.

At the initial phase of HIV epidemic in Thailand, subtype B was predominantly found in injection drug user. However, the circulated HIV-1 strains were inconsistence; CRF01_AE became the strain that commonly found in newly infected patient both from injecting drug use and sexual transmission. The study of circulating strains of HIV-1 in all geographic region of Thailand; rely on *env* variation, during 1995-2004 showed the 97.3% prevalence of subtype CRF01_AE and 2.7% of subtype B. Recently, there was investigation about HIV-1 epidemic in northern Thailand based on genotyping of *pol* gene reveled that subtype CRF01_AE has been found 94.8%, subtype B 2.9%, and subtype C 0.5%. In addition, the study of HIV-1 in Bangkok and the nearly provinces according to *pol* gene exposed the emergence of 86.9% for subtype CRF01_AE, 8.6% for subtype B, and 4.5% were the other recombinant strains.

In this study, total of 35 samples from 675 samples were randomly selected for HIV-1 nearly full-length genomic sequencing study. A total of 675 plasma samples from year 2009-2011 sent to laboratory for HIV drug resistant genotyping assay were collected for the study. Most samples were collected derived from

antiretroviral treated HIV infected patients from four area of Thailand. The analysis of *pol* gene demonstrated that the majority circulating subtypes during 2009-2011 were CRF01_AE (94%) which were concordance to other studies (53, 54, 55). Subtype B was found secondly and followed by other subtypes such as subtype C, CRF01_AE/B, CRF02_AG, and other recombinant strains. Subtypes CRF01_AE and B C the most globally subtypes, common form Myanmar (68).

Nucleotide sequences covered HIV *gag, pol* and *env* (8,764 bp.) regions, nearly full-length HIV-1 genomic sequences, analysis of 35 samples showed that all sequences were CRF01_AE linked to CRF01_AE reference sequences from Thailand. There were 3 recombinant strains (8.6%) between subtypes CRF01_AE and B with CRF01_AE backbone in *gag* & *env* genes and B sequences in *pol* gene at position 2267-3519. There was a report of CRF01_AE/B indicating that this recombinant virus was heterosexually transmitted in northern Thailand from Kwanjai et.al in 2004 and HIV CRF01/B recombinants were reported globally including CRF15_01B, CRF33_01B, CRF34_01B, CRF48_01B, CRF51_01B, CRF55_01B, CRF58_01B, CRF59_01B, CRF67_01B, CRF68_01B, CRF74_01B and CRF76_01B. CRF15_01B was first reported from Thailand that sequence show subtype CRF01_AE with only the env region being derived from subtype B (56). CRF33_01B was first reported from Malaysia that two short subtype B segments were inserted into the gag-RT region in a backbone of CRF01_AE (57). CRF34_01B was first reported from Thailand that sequence show subtype CRF01_AE, but with segments pol and gp41 showed subtype B (58). CRF48_01B was first reported from Malaysia (59). CRF51_01B was first reported from Thailand that two short subtype B segments were inserted into a backbone of CRF_01AE (60). CRF55_01B was first reported from China that separated from MSM patients (61). CRF58_01B was first reported from Malaysia that that separated from IV drug users (62). CRF59_01B was first reported from China that comprised of CRF01_AE and subtype B, with two recombination breakpoints in the pol and vpu-env regions (63). CRF67_01B was first reported from China that separated from MSM patients (64). CRF68_01B was first reported from China (65). CRF74_01B was first reported from Malaysia that were composed of CRF01_AE and subtype B', with eight breakpoints dispersed in the gag-pol and nef regions (66).

CRF76_01B was first reported from Japan that were composed of CRF01_AE and subtype B with seven breakpoints in the gag, pol, vif, and env regions (67). All 3 samples were compared to other CRF01B recombinant strains from HIV database. They were clustered with CRF34_01B and CRF52_01B from Thailand and Malaysia. (Sanders-Buell et al. 2007 and Liu et al. 2012.)

The result of drug resistant showed that DRMs to NNRTIs resistant was found predominantly followed by DRMs to NRTIs. The prevalence of DRMs to NNRTIs (72.23%) and NRTIs (61.67%) year 2009-2011 were concordance to study of Sanguansittianant et al and other countries but higher than the study of Sangkanuparph et al. (58, 49, 50, 51, 52, 53, 54,55). Moreover, this study showed prevalence of DRMs to PIs at 1.9%. Among DRMs to NRTIs, M184V was found the most common (57.71%) which conferred to 3TC or FTC resistant. The prevalence of Q151M which confer to broad NRTIs cross resistant was 13.09%. During 2009-2011; DRMs to ABC, AZT, D4T, ddI and TDF were continuously decreasing but only DRMs to 3TC and FTC were still stable with high rate of resistant. The most commonly detected of DRMs to NNRTIs included K101E (17.63%), K103N (25.92%), Y181C (35.25%), G190A (20.95%) and F227C/L (1.88%) which were concordance to previous studies (55, 56, 53, 57), similar to what has been described in subtype B infection. For PIs, the prevalence of DRMs to PIs during 2009-2011 was 5.7%. The lack of increasing DRMs to during 2009-2011, may be resulted from introduction of protease inhibitor with combination therapy. Among DRMs to PIs, the most commonly observed mutation codons during 2009-2011 were V82A (13.98%), M46I (1.74%) and I54M (1.31%). V82A and M46I mutation codons were detected at higher percentages than previous studies (55, 56). Moreover, the frequency of L90M during 2009-2011 was found less compared to other studies (58, 55). All other DRMs to protease inhibitors showed increasing frequency rate during 2009-2011. HIV-1 drug resistance mutation related with subtype of non - B subtype has rarely reported resistance on basic single drug (NRTI backbones) but had reported for specific drug class.the. NNRTI resistance-associated mutation V106M is more common in subtype C than subtype B viruses. NRTIs resistance combinations of thymidine analogue mutations (TAMs) such as codon M41L and T215Y the most common in subtypes B treated with zidovudine.

This study was found that major subtype of HIV-1 is subtype CRF01_AE and B, which corresponding the HIV epidemiology of Thailand. The resulting of molecular epidemiology of HIV-1 and DRMs frequency is also important to the development of drugs useful for the selection of drug therapies. In each of these areas, and intracking the evolution of the HIV-1 pandemic, differences among subtypes continue to play an important role.

REFERENCES

1. Barré-Sinouss,. F.; Chermann, J.C.; Rey, F.; Nugeyre, M.T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983; 220: 868–871.
2. Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science; 1984: 224(4648), 497–500.
3. UNAIDS, 2010. Global Report: UNAIDS Report on the Global AIDS Epidemic 2010. Joint United Nations Programme on HIV/AIDS (UNAIDS).
4. Bureau of Epidemiology, Department of Disease Control Ministry of Public Health, HIV virus disease cases and deaths reported during1984-November 2011 cited. Available from: http://www.boe.moph.go.th/files/report/20111206_93584707.
5. Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemée V, et al. A new human immunodeficiency virus derived from gorillas. Nat Med. 2009; 15(8): 871–72.
6. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. AIDS 2006; 20(16): W13-23.
7. Hemelaar J, Gouws E, Ghys PD, Osmanov S; WHO-UNAIDS Network for HIV Isolation and Characterisation. Global trends in molecular epidemiology of HIV-1 during 2000-2007. AIDS. 2011; 25(5): 679-89.
8. Salemi M, Strimmer K, Hall WW, Duffy M, Delaporte E, Mboup S, et al. Dating the common ancestor of SIVcpz and HIV-1 group M and the origin of HIV-1 subtypes using a new method to uncover clock-like molecular evolution. FASEB J. 2001; 15(2), 276–8.

9. Sharp PM, Bailes E, Gao F, Beer BE, Hirsch VM, Hahn BH. Origins and evolution of AIDS viruses: estimating the time-scale. Biochem Soc Trans. 2000; 28(2): 275-82.
10. Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, et al. Timing the ancestor of the HIV-1 pandemic strains. Science. 2000; 288(5472): 1789–96.
11. Hu W, Temin H. Retroviral recombination and reverse transcription. Science.1990; 250(4985): 1227–33.
12. Shriner D, Rodrigo AG, Nickle DC, Mullins JI. Pervasive genomic recombination of HIV-1 in vivo. Genetics. 2004; 167(4): 1573–83.
13. Preston B, Poiesz B, Loeb L. Fidelity of HIV-1 reverse transcriptase. Science. 1988; 242(4882): 1168–71.
14. Roberts JD, Bebenek K, Kunkel TA. The accuracy of reverse transcriptase from HIV-1. Science. 1988: 242(4882): 1171–3.
15. Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature. 1995; 373(6510): 117–22.
16. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature. 1995; 373(6510): 123–6.
17. Ross LL, Weinberg WG, DeJesus E, Fischl MA, Horton JH, Pappa KA, et al. Impact of low abundance HIV variants on response to ritonavir-boosted atazanavir or fosamprenavir given once daily with tenofovir/emtricitabine in antiretroviral-naive HIV-infected patients. AIDS Res. Hum. Retrov. 2010; 26(4): 407–17.
18. Carvajal-Rodríguez A, Posada D, Pérez-Losada M, Keller E, Abrams EJ, Viscidi RP, et al. Disease progression and evolution of the HIV-1 env gene in 24 infected infants. Infect. Genet. Evol. 2008; 8(2): 110–20.
19. Lemey P, Kosakovsky Pond SL, Drummond AJ, Pybus OG, Shapiro B, Barroso H, et al. Synonymous substitution rates predict HIV disease progression as a result of underlying replication dynamics. PLoS Comput. Biol. 2007; 3(2): 282–92.

20. Peeters M, Vincent R, Perret JL, Lasky M, Patrel D, Liegeois F, et al. Evidence for differences in MT2 cell tropism according to genetic subtypes of HIV-1: syncytium-inducing variants seem rare among subtype C HIV-1 viruses. Journal of Acquired Immune Deficency Syndrome and Human Retrovirology. 1999; 20: 115-21.
21. Kaleebu P, French N, Mahe C, Yirrell D, Watera C, Lyagoba F, et al. Effect of human immunodeficiency virus (HIV) type 1 envelope subtypes A and D on disease progression in a large cohort of HIV-1-positive persons in Uganda. J. Infect. Dis. 2002; 185: 1244–50.
22. Vasan A, Renjifo B, Hertzmark E, Chaplin B, Msamanga G, Essex M, et al. Different rates of disease progression of HIV type 1 infection in Tanzania based on infecting subtype. Clin. Infect. Dis. 2006; 42: 843–52.
23. Palmer S, Alaeus A, Albert J, Cox S. Drug susceptibility of subtypes A, B, C, D, and E human immunodeficiency virus type 1 primary isolates. AIDS Res Hum Retroviruses. 1998; 14: 157–62.
24. Llibre JM, Schapiro JM, Clotet B. Clinical implications of genotypic resistance to the newer antiretroviral drugs in HIV-1-infected patients with virological failure. Clin. Infect. Dis. 2010; 50(6): 872–81.
25. Peterlin BM, Trono D. Hide, shield and strike back: how HIV-infected cells avoid immune eradication. Nat Rev Immunol. 2003; 3(2): 97-107.
26. Gallo RC, Montagnier L. The Discovery of HIV as the Cause of AIDS. New England Journal of Medicine. 2003; 349(24): 2283-5.
27. Levy JA, editors, Dicovery and Nature of the HIV Virion. In: HIV and the Pathogenesis of AIDS. Washington, D.C., USA; ASM Press: 1994: 2-5.
28. HIV Virus Research [online]. cited. Available from: http://www.eenzyme.com/hivresearchtools.aspx.
29. HIV Replication Cycle [online]. cited. Available from: http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/pages/hivreplicationcycle.aspx.
30. Peterlin BM, Trono D. Hide, shield and strike back: how HIV-infected cells avoid immune eradication. Nat Rev Immunol. 2003; 3(2): 97-107.

31. Kirchhoff F. Is the high virulence of HIV-1 an unfortunate coincidence of primate lentiviral evolution? Nat Rev Microbiol. 2009; 7(6): 467-76.
32. Benjamin s. weeks, Alcmo IE. AIDS THE BIOLOGY BASIC. In: Book AIDS THE BIOLOGY BASIC. FIFTH EDITION ed. Sudbury Massachusetts: Jones and Bartlett; 2010. 46-54 p.
33. Devita VT, Hellman JS, Rosenberg SA. AIDS Etiology, Diagnosis, Treatment and Prevention. In: Book AIDS Etiology, Diagnosis, Treatment and Prevention. Fourth Edition ed. Philadelphia New York: Lippicott-Raven; 1997. 29-66 p.
34. NIAID. HIV Replication Cycle [online]. cited. Available from: http://www.niaid.nih.gov/topics/HIVAIDS/Understanding/Biology/pages/hivreplicationcycle.aspx.
35. Vallari A, Holzmayer V, Harris B, Yamaguchi J, Ngansop C, Makamche F, et al. Confirmation of putative HIV-1 group P in Cameroon. J Virol. 2011; 85(3): 1403-7.
36. Sharp PM, Hahn BH. The evolution of HIV-1 and the origin of AIDS. Philos Trans R SocLond B Biol Sci. 2010; 365(1552): 2487-94.
37. Thomson MM, Perez-Alvarez L, Najera R. Molecular epidemiology of HIV-1 genetic forms and its significance for vaccine development and therapy. Lancet Infect Dis. 2002; 2(8): 461-71.
38. Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The Challenge of HIV-1 Subtype Diversity. New England Journal of Medicine. 2008; 358(15): 1590-602.
39. WHO. Adults and children estimated to be living with HIV, 2011 [online]. cited. Available from: http://gamapserver.who.int/mapLibrary/Files/Maps/HIV_all_2011.png.
40. WHO. Global sumary of the AIDS epidemic 2011 [online]. cited. Available from: http://www.who.int/hiv/data/2012_epi_core_en.png.
41. Auswinporn S, Jenwitheesuk E, Vibhagool A, Sookpranee M, Leechawengwong M, Chantratita W. Genotypic subtyping of the HIV-1 polymerase gene in 30 naive patients from Thailand. Southeast Asian J Trop Med Public Health. 2001; 32(2): 346-50.

42. Wirachsilp P, Kantakamalakul W, Foongladda S, Chuenchitra T, Kohriangudom S, Athipanyasilp N, et.al. Surveillance of subtype and genetic variation of the circulating strains of HIV-1 in Thailand. Southeast Asian J Trop Med Public Health. 2007; 38(5): 814-27.
43. Praparattanapan J, Tragoolpua Y, Pathom-aree W, Kotarathitithum W, Chaiwarith R, Nuntachit N, Sirisanthana T, Supparatpinyo K. Current molecular epidemiology and recombination of HIV type 1 subtypes in northern Thailand. AIDS Res Hum Retroviruses. 2011; 27(11): 1201-6.
44. Merigan TC Jr, Bartlett JG, Bolognesi D. Control and prevention. In: Pine JW Jr, Mullen ML, Carley PJ, editors. Textbook of AIDS medicine. 2nd edition. Baltimore, USA: Williams & Wilkins; 1999: 689-780 p.
45. Zeichner SL, Read JS, editors. Antiretroviral theraphy. In. Textbook of pediatric HIV case. 1st edition. Cambridge, United Kingdom: Cambridge university press; 2005: 287-302, 334-48 p.
46. Clavel F, Hance AJ. HIV Drug Resistance. N Engl J Med. 2004; 350(10): 1023-35.
47. Nadai Y, Eyzaguirre LM, Constantine NT, Sill AM, Cleghorn F, Blattner WA, et al. Protocol for nearly full-length sequencing of HIV-1 RNA from plasma. PLoS One. 2008; 3(1): e1420.
48. Li L, Liang S, Chen L, Liu W, Li H, Liu Y, et al. Genetic characterization of 13 subtype CRF01_AE near full-length genomes in Guangxi, China. AIDS Res Hum Retroviruses. 2010; 26(6): 699-704.
49. Pitisuttithum P, Rerks-Ngarm S, Connell R, Kim J, Excler J-L. An HIV Vaccine for South-East Asia-Opportunities and Challenges. Vaccines. 2013; 1(3): 348-66.
50. Sanguansittianant S, Nooroon N, Phaengchomduan P, Ammaranond P. Trends in Prevalence of HIV-1 Drug Resistance in Thailand 2009–2010. J Clin Lab Anal. 2013; 27(5): 346-53.
51. Manosuthi W, Thongyen S, Nilkamhang S, Manosuthi S, Sungkanuparph S. HIV-1 drug resistance-associated mutations among antiretroviral-naive thai patients with chronic HIV-1 infection. J Med Virol. 2013; 85(2): 194-9.

52. Coetzer M, Westley B, Delong A, Tray C, Sophearin D, Nerrienet E, et al. Extensive drug resistance in HIV-infected Cambodian children who are undetected as failing first-line antiretroviral therapy by WHO 2010 guidelines. AIDS Res Hum Retroviruses. 2013; 29(7): 985-92.

53. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Chantratitra W. Tenofovir resistance among HIV-infected patients failing a fixed-dose combination of stavudine, lamivudine, and nevirapine in a resource-limited setting. AIDS Patient Care & STDs. 2007 Oct; 21(10):711–714.

54. Sanguansittianant S, Nooroon N, Phaengchomduan P, Ammaranond P. Trends in prevalence of HIV-1 drug resistance in Thailand 2009-2010. J Clin Lab Anal. 2013 Sep;27(5):346-53.

55. Sukasem C, Churdboonchart V, Sirisidthi K, Riengrojpitak S, Chasomba S, Watitpun C, et al.Genotypic resistance mutations in treatment-naive and treatment-experienced patients under widespread use of antiretroviral drugs in Thailand: implications for further epidemiologic surveillance. Jpn J Infect Dis. 2007; 60(5):284–289.

56. Tovanabutra S1, Watanaveeradej V, Viputtikul K, De Souza M, Razak MH, Suriyanon V, Jittiwutikarn J, et al. A new circulating recombinant form, CRF15_01B, reinforces the linkage between IDU and heterosexual epidemics in Thailand. AIDS Res Hum Retroviruses. 2003 Jul;19(7):561-7.

57. Tee KK1, Li XJ, Nohtomi K, Ng KP, Kamarulzaman A, Takebe Y. Identification of a novel circulating recombinant form (CRF33_01B) disseminating widely among various risk populations in Kuala Lumpur, Malaysia. J Acquir Immune Defic Syndr. 2006 Dec 15;43(5):523-9.

58. Tovanabutra S1, Kijak GH, Beyrer C, Gammon-Richardson C, Sakkhachornphop S, Vongchak T, Jittiwutikarn J, et al. Identification of CRF34_01B, a second circulating recombinant form unrelated to and more complex than CRF15_01B, among injecting drug users in northern Thailand. AIDS Res Hum Retroviruses. 2007 Jun;23(6):829-33.

59. Li Y1, Tee KK, Liao H, Hase S, Uenishi R, Li XJ, Tsuchiura T, et al. Identification of a novel second-generation circulating recombinant form (CRF48_01B) in Malaysia: a descendant of the previously identified CRF33_01B. J Acquir Immune Defic Syndr. 2010 Jun;54(2):129-36.

60. Ng OT1, Eyzaguirre LM, Carr JK, Chew KK, Lin L, Chua A, Leo YS, et al. Identification of new CRF51_01B in Singapore using full genome analysis of three HIV type 1 isolates. AIDS Res Hum Retroviruses. 2012 May;28(5):527-30.

61. Han X1, An M, Zhang W, Cai W, Chen X, Takebe Y, Shang H. Genome Sequences of a Novel HIV-1 Circulating Recombinant Form, CRF55_01B, Identified in China. Genome Announc. 2013 Jan;1(1)

62. Chow WZ1, Takebe Y2, Syafina NE1, Prakasa MS1, Chan KG3, Al-Darraji HA1, Koh C1, et al. A newly emerging HIV-1 recombinant lineage (CRF58_01B) disseminating among people who inject drugs in Malaysia. PLoS One. 2014 Jan 22;9(1)

63. Han X1, An M, Zhang W, Zhao B, Chu Z, Takebe Y, Shang H. Genome, et al. Sequences of a Novel HIV-1 Circulating Recombinant Form (CRF59_01B) Identified among Men Who Have Sex with Men in Northeastern China. Genome Announc. 2013 Jun 27;1(3)

64. Wu J1, Meng Z, Xu J, Lei Y, Jin L, Zhong P, Han R, Su B, et al. New emerging recombinant HIV-1 strains and close transmission linkage of HIV-1 strains in the Chinese MSM population indicate a new epidemic risk. PLoS One. 2013;8(1)

65. Wu J1, Meng Z, Xu J, Lei Y, Jin L, Zhong P, Han R, Su B. New emerging recombinant HIV-1 strains and close transmission linkage of HIV-1 strains in the Chinese MSM population indicate a new epidemic risk. PLoS One. 2013;8(1)

66. Cheong HT1, Chow WZ1, Takebe Y2, Chook JB1, Chan KG3, Al-Darraji HA1, Koh C1, et al. Genetic Characterization of a Novel HIV-1 Circulating Recombinant Form (CRF74_01B) Identified among Intravenous Drug Users in Malaysia: Recombination History and Phylogenetic Linkage with Previously Defined Recombinant Lineages. PLoS One. 2015 Jul 21;10(7)

67. Ogawa S1,2, Hachiya A1,3, Hosaka M1, Matsuda M1, Ode H1, Shigemi U1, Okazaki R1, et al. A Novel Drug-Resistant HIV-1 Circulating Recombinant Form CRF76_01B Identified by Near Full-Length Genome Analysis. AIDS Res Hum Retroviruses. 2016 Mar;32(3):284-9

68. Zhou Y, Liang Y, Pang W, Qin W, Hong Yao Z, Chen X, et al. Diverse forms of HIV-1 among Burmese long-distance truck drivers imply their contribution to HIV-1 cross-border transmission. BioMed Central . 2014; 14(463):1,471–2,334.

69. University S. HIV Drug Resistance Database [internet] 2008 [updated July 31, 2008; cited 2016 January 25]. Available from : http://hivdb.stanford.edu .

70. AIDSinfo Guidelines for the use of antiretroviral therapy agents in HIV-1 infected pediatric, adults and pregnant person [internet]. Available from: http://thaiaidssociety.org

APPENDIX

Solutions

1. **0.5 TBE**

10X TBE Buffer Liquid	50	ml
Distill water	950	ml

2. **1.0% agarose gel**

0.5x TBE	100	ml
Agarose base	1.0	g

3. **Gel Red gel stain mixture**

DNAse RNAse free water	90	ml
1% NaCl	10	ml
Gel RED^{TM} (Biotium)	30	µl

4. **Deoxynucleotide triphosphate (10 mM dNTPs) mixture**

dATP	100	µl
dCTP	100	µl
dGTP	100	µl
dTTP	100	µl

5. **6X loading dye**

Bromophenol	0.25	g
Sucrose	50	g
1M Tris HCL	1	ml
Distill water	100	ml

6. **Phosphate buffer saline (PBS)**

KCl	0.2	g
NaCl	8	g
Na_2HPO_4	1.44	g
KH_2PO_4	0.24	g

Add all components in 800 ml of distilled water and adjust pH to 7.4 with 1M HCl. Add distilled water to 1,000 ml and autoclave at 121 ^{o}C for 20 minutes

BIOGRAPHY

NAME	Mr. Nattapol Narong
DATE OF BIRTH	23 July 1986
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTE ATTENDED	Mahidol University, 2006-2009: Bachelor of Science (Medical Technology) Mahidol University, 2011-2016 Master of Science (Medical Microbiology)
RESEARCH-GRANT	The Siriraj Graduate Thesis Scolarship
HOME ADDRESS	80/3, Authongmakarmthou Road Warsink, Chainat 17120 Tel 087-6775497 E-mail: nattapon_237@hotmail.com