APPENDICES

Appendix A: The nucleotide sequencing results of 40 clinical specimens from in-house HIV-1 genotypic drug resistant assay

Sample No.	Sample ID.	Sequence (5'→ 3')
Consensus	-	A GAA ATR GTT ATC TAT CAA TAC **ATG** GAT GAC TTG TAT GTA GGA TC
S1	01	A GAA ATG GTT ATC TAT CAA TAT **ATG** GAT GAC TTG TAT GTA GGA TC
S2	02	A GAA ATA GTT ATC TAC CAG TAT **GTG** GAT GAC TTG TAT GTA GGA TC
S3	03	A GAA ATR GTT ATC TAT CAA TAC **GTG** GAC GAC TTG TAT GTA GGA TC
S4	04	A GAA ATA ATT ATM TGT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S5	05	A GAC ATG RTT ATC TGT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S6	06	A GAA ATG GTT ATC TGT CAA TAC **GTA** GAT GAC TTG TAT GTA GGA TC
S7	07	A GAA ATA GTT ATC TGT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S8	08	A GAA ATG GTT ATC TAT CAA TAC **ATA** GAT GAC TTG TAT GTA GCA TC
S9	09	A GAA ATG GTT ATT TGT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S10	10	A GAA ATG GTT ATC TGT CAA TAC **GTG** GAT GAC TTG TAT GTA GGG TC
S11	11	A GAA ATA GAT ATC TAT CAA TAT **GTG** GAT GAT TTG TTT GTA GGA TC
S12	12	A GAA ATA ATT ATC TGT CAA TAC **GTG** GAT GAT TTG TAT GTA GGA TC
S13	13	A GAA ATG GTC ATC TAT CAA TAC **ATG** GAT GAC TTG TAT GTA GGA TC
S14	14	A GAC ATA GTT ATA TAT CAG TAC **GTG** GAT GAT TTG TAT GTA GCA TC
S15	15	A GAA ATA GTT ATC TGT CAA TAC **GTG** GAT GAC TTG TAT GTA AGT TC
S16	16	A GAA ATG ATT ATC TAT CAA TAC **GTG** GAT GAC TTG TAT GTA GCA TC
S17	17	A GAA ATG ATT ATC TGT CAA TAC **GTR** GAT GAC CTG TAT GTA GGA TC
S18	18	A GAA ATG GTT ATC TRT CAA TAC **GTA** GAT GAC TTG TAT GTA GGA TC
S19	19	A GAA ATA GTT ATC TGT CAA TAC **GTA** GAT GAT TTA TAT GTA GGA TC

Appendix A (Continued)

No.	Sample ID.	Sequence (5'→ 3')
S20	20	A GAA ATA GTT ATC TAT CAA TAC **RTG** GAT GAC TTG TGT GTA GGG TC
S21	21	A GAA MTG GTT ATM TRT CAA TAC **ATG** GAT GAC TTG TAT RTA GSA TC
S22	23	A GAA ATG GTT ATC TGT CAA TAC **ATG** GAT GAC TTG TAT GTA GGA TC
S23	24	A GA*C* ATA GTT ATC TGT CAA TAC **GTG** GAT GAT CTA TAT GTT GGA TC
S24	25	A GA*C* ATA GTT ATY TAT CAA TAY **GTR** GAT GAC TTG TAT GTA GGA TC
S25	26	A GAA ATG GTT ATC TGT CAA TAC **GTG** GAT GAC TTR TAT GTA GGA TC
S26	27	A GAA ATG GTT ATC TAT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S27	28	A GAA ATA GTT ATC TAT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S28	29	A GRA ATA GTT ATC TAT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S29	30	A GAA ATA GTT ATC TAT CAA TAC **GTG** GAT GAC TTG TAT GTA GGG TC
S30	31	A GAA ATT GTT ATC TGT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S31	32	A GAA ATA RTT ATC TGT CAA TAT **GTG** GAT GAC TTG TAT GTA GGA TC
S32	33	A GAA ATG GTT ATC TAT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S33	34	A GAG ATG ATT ATC TAT CAA TAC **GTG** GAT GAC TTG TAT GTA GGA TC
S34	35	A GAA ATA GTY ATM TRT CAA TAC **ATG** GAT GAC TTG TAT GTA GGA TC
S35	36	A GAA ATG GTT ATC TAT CAA TAC **ATG** GAT GAC TTG TAT GTA GGA TC
S36	37	A GAA ATG GTT ATC TAT CAA TAY **GTG** GAT GAY TTG TAT GTA GCA TC
S37	38	A GAA ATG ATT ATC TGC CAG TAC **GTG** GAC GAT TTG TAT GTA GGA TC
S38	39	A GAA ATA ATT ATC TAT CAA TAC **ATA** GAT GAC TTG TAT GTA GCA TC
S39	40	A GAA ATG GTT ATC TAT CAA TAT **GTG** GAT GAC TTG TAT GTA AGC TC
S40	41	A GAA ATA GAG RTC TAT CAA TAC **ATG** GAT GAC TTG TAT GTA GGA TC

Appendix B: Important and useful terminology

Assay performance: the ability or efficacy of the assay to do something well. The action or manner of carrying out an activity, piece of work.

Enzyme-linked Immunosorbent Assay (ELISA): a sensitive immunoassay that uses an enzyme linked to an antibody or antigen as a marker for the detection of a specific protein, especially an antigen or antibody.

Genotype: the genetic makeup, as distinguished from the physical appearance, of an organism or a group of organisms.

HIV-1 drug treatment failure: there are 3 categories to define treatment failure: virological failure, immunological failure and clinical failure. Virological failure is the most sensitive and accurate way to diagnose early treatment failure, plasma viral load assay is an important tool. Virological failure is defined as viral load > 1,000 copies/ml in clinical practice. Criteria for virological failure include: viral load > 1,000 copies/ml after 6 months of receiving ART with good adherence, or rebound of viral load to > 1,000 copies/ml in any duration after achieving viral load < 50 copies/ml. Immunological failure is considered when there is a decrease or delayed increase of CD_4 T-cell count after ART, however immunological failure is not sensitive. Criteria for immunological failure include: CD_4 T-cell count increase < 50 cell/mm^3 after a year of ART, absolute CD_4 T-cell count decrease > 30% or percent CD_4 decrease > 3% from the highest level previously gained, CD_4 T-cell count

decrease to the level lower than pre-ART level. Clinical failure is the most delayed method to diagnose treatment failure. Patients usually have virological and immunological failure for a period of time before clinical failure occurs. Clinical failure may manifest as clinical as clinical relapse of prior opportunistic infection or occurrence of a new opportunistic infection. Immune reconstitution inflammatory syndrome (IRIS) needs to be excluded before a diagnosis of clinical failure.

M184V mutation: the nucleotide substitution, from A to G, at the position 3099 HXB2 in the *pol* region resulting in the changes of amino acid from methionine (ATG) to valine (GTG) at codon 184 of the reverse transcriptase (RT) enzyme. This mutation is selected by therapeutic regimens containing Lamivudine (2',3'-dideoxy-3'-thiacytidine, 3TC) and confers a loss of susceptibility, range from 100- to 1,000-fold, to this drug. Selection of this mutation by 3TC occurs rapidly compared to the development of resistance to other drugs.

Mutant: an individual, organism, or new genetic character arising or resulting from an instance of mutation, which is a base-pair sequence change within the DNA of a gene or chromosome of an organism resulting in the creation of a new character or trait not found in the wild type. The natural occurrence of genetic mutations is integral to the process of evolution. The study of mutants is an integral part of biology, by understanding the effect that a mutation in a gene has it is possible to establish the normal function of that gene. In some organisms mutants can be created by gene targeting to assess the function of any given gene. This experimental approach is called reverse genetics.

Optimization: the design and operation of a system or process to make it as good as possible in some defined sense.

Phenotype: the observable characteristics of an organism, such as shape, size, color, and behavior, that results from the interaction of its genotype (total genetic makeup) with the environment. Drug-resistant phenotype of the virus is determined by the reduction of the drug susceptibility compared with the susceptibility of wild-type viruses. The concentrations of drug required to inhibit virus replication by 50% (IC_{50}) or 90% (IC_{90}) are the most commonly used measures of drug susceptibility.

Point mutation: a mutation that involves a single nucleotide as a result of nucleotide deletion, substitution, or the insertion of an additional nucleotide.

Polymerase Chain Reaction (PCR): a technique to exponentially amplify a small quantity of a specific nucleotide sequence in the presence of template sequence, two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA, and a thermostable (*Taq*) DNA polymerase. The PCR cycle involving template denaturation, primer annealing, and the extension of the annealed primers by DNA polymerase until enough copies are made for further analysis.

Primary mutations: (also known as major mutations) mutations that reduce drug susceptibility by themselves.

Primary resistance: transmission of drug-resistance variant, and some evidence that transmitted resistance may compromise response to first-line therapy. In some case, the presence of resistance in an apparently drug-naïve patient may in fact reflect previous undisclosed therapy.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR): RT-PCR involves two steps: the RT reaction and PCR amplification. RNA is first reverse transcribed into cDNA by the reverse transcriptase. The cDNA, then, acts as templates for subsequent PCR amplification using primers specific for one or more genes. RT-PCR can also be carried out as the one-step RT-PCR in which all reaction components are mixed in one tube prior to starting the reactions.

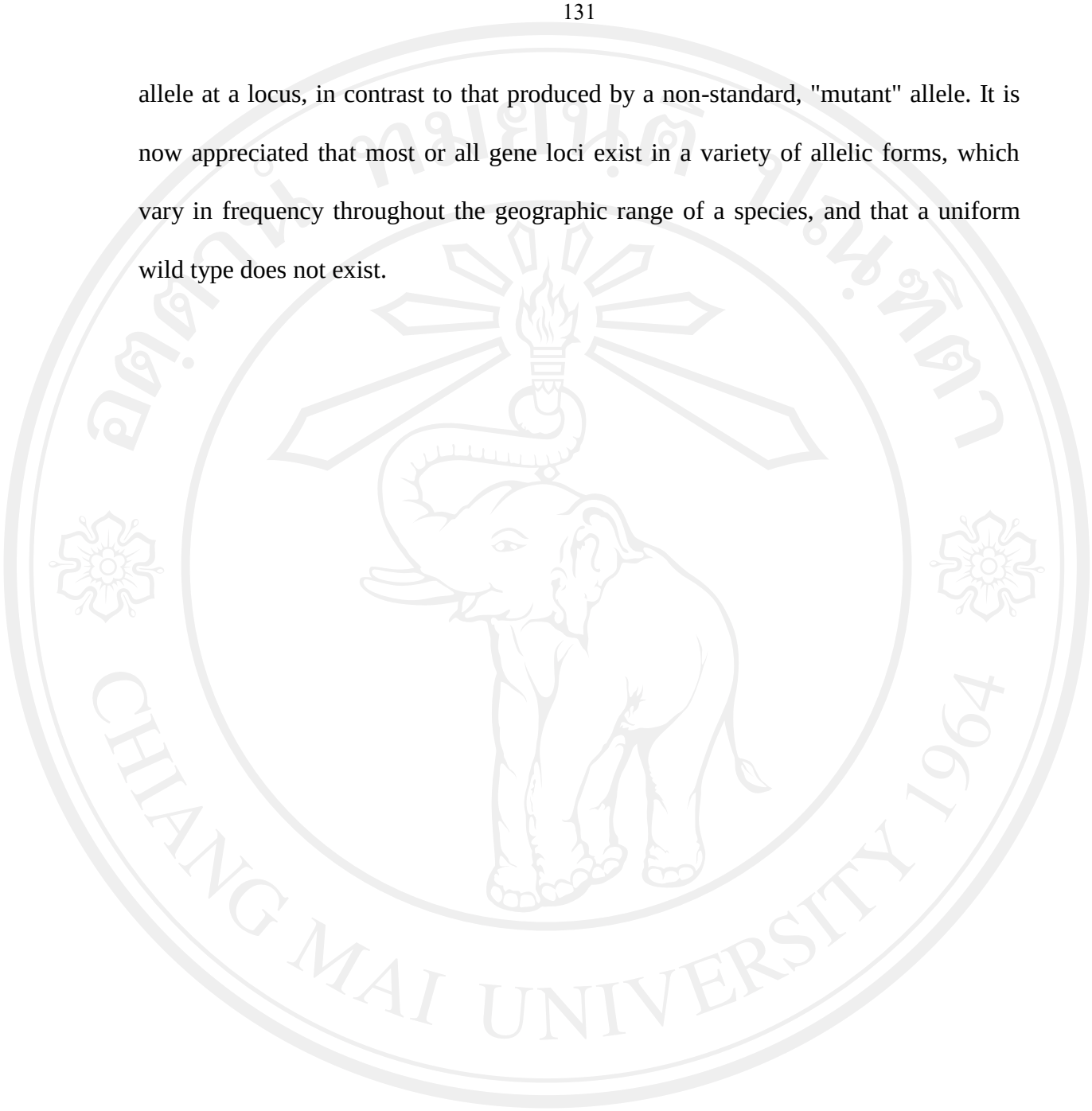
Secondary mutations: (also known as minor mutations) mutations that reduce drug susceptibility or improve the replicative fitness of isolates with a primary mutation.

Secondary resistance: the resistance arises during or after drug therapy.

Viral fitness: the adaptability of a virus to its environment in term of replicative capacity. Variants with higher fitness can out-compete other variants of lower fitness as measured by tissue culture assay, including viral growth kinetics, single-cycle infection and growth competition.

Wild type: the phenotype of the typical form of a species as it occurs in nature. Originally, the wild type was conceptualized as a product of the standard, "normal"

allele at a locus, in contrast to that produced by a non-standard, "mutant" allele. It is now appreciated that most or all gene loci exist in a variety of allelic forms, which vary in frequency throughout the geographic range of a species, and that a uniform wild type does not exist.



Appendix C: Amino acid codes

Amino acid	3 –Letter code	1 – Letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Appendix D: Standard genetic code

FIRST LETTER	SECOND LETTER T	SECOND LETTER C	SECOND LETTER A	SECOND LETTER G	THIRD LETTER
T	TTT = Phe = F	TCT = Ser = S	TAT = Tyr = Y	TGT = Cys = C	T
	TTC = Phe = F	TCC = Ser = S	TAC = Tyr = Y	TGC = Cys = C	C
	TTA = Leu = L	TCA = Ser = S	TAA ≠ STOP = O	TGA ≠ STOP = X	A
	TTG = Leu = L	TCG = Ser = S	TAG ≠ STOP = U	TGG = Trp = W	G
C	CTT = Leu = L	CCT = Pro = P	CAT = His = H	CGT = Arg = R	T
	CTC = Leu = L	CCC = Pro = P	CAC = His = H	CGC = Arg = R	C
	CTA = Leu = L	CCA = Pro = P	CAA = Gln = Q	CGA = Arg = R	A
	CTG = Leu = L	CCG = Pro = P	CAG ≠ Gln = Z	CGG = Arg = R	G
A	ATT = Ile = I	ACT = Thr = T	AAT = Asn = N	AGT = Ser = S	T
	ATC = Ile = I	ACC = Thr = T	AAC ≠ Asn = B	AGC = Ser = S	C
	ATA ≠ Ile = J	ACA = Thr = T	AAA = Lys = K	AGA = Arg = R	A
	ATG = Met = M	ACG = Thr = T	AAG = Lys = K	AGG = Arg = R	G
G	GTT = Val = V	GCT = Ala = A	GAT = Asp = D	GGT = Gly = G	T
	GTC = Val = V	GCC = Ala = A	GAC = Asp = D	GGC = Gly = G	C
	GTA = Val = V	GCA = Ala = A	GAA = Glu = E	GGA = Gly = G	A
	GTG = Val = V	GCG = Ala = A	GAG = Glu = E	GGG = Gly = G	G

Appendix E: Reagents preparation

1. Agarose gel electrophoresis

1% Agarose gel

Agarose powder	1 g
1X TE buffer	100 ml

Ethidium bromide 5 mg/ml: 10 ml

Ethidium bromide	50 mg
Sterile deionized water	10 ml

Tris-Borate / EDTA Electrophoresis buffer (TBE) pH 8.3

Stock 10X buffer: 1,000 ml

Trizma base	121.1 g
Boric acid anhydrous	55.6 g
$Na_2EDTA.{}_2H_2O$	3.7 g
Add sterile deionized water up to	1,000 ml

Working 1X buffer: 1,000 ml

10X TBE buffer	100 ml
Sterile deionized water	900 ml

6X Loading buffer: 100 ml

Bromophenol blue	0.25 g
Xylene cyanol FF	0.25 g
Glycerol	30 ml
1X TBE	70 ml

2. Oligonucleotide ligation assay (OLA)

Phosphate buffer saline (PBS) pH 7.2

Stock 10X PBS: 1,000 ml

NaCl	25 g
Na_2HPO_4	12 g
NaH_2PO_4	2 g
Add sterile deionized water up to	1,000 ml

Working 1X PBS: 1,000 ml

10X PBS	100 ml
Sterile deionized water	900 ml

0.5% Bovine serum albumin (BSA) blocking solution: 500 ml

BSA	2.5 g
1X PBS	500 ml

Ligation buffer (no NAD)

10X Ampligase reaction buffer: 10 ml

1M Tris-HCl, pH 8.5	2 ml
1M KCl	2.5 ml
1M $MgCl_2$	1 ml
1% Triton X-100	1 ml
Sterile deionized water	3.5 ml

1M KCl: 100 ml

KCl	7.456 g
Add sterile deionized water up to	100 ml

1M MgCL2: 100 ml

MgCL2. 6H2O	20.33 g
Add sterile deionized water up to	100 ml

1X NaOH wash: 100 ml

10N NaOH	100 µl
Tween 20	50 µl
Add sterile deionized water up to	100 ml

Stop Ligation solution

Stop Ligation solution (0.1M EDTA/0.1% Triton-X 100): 50 ml

0.5 M EDTA	10 ml
1% Triton-X 100	5 ml
Ultrapure water (Gibco)	35 ml

0.5 M Ethylenediamine tetraacetic acid (EDTA): 100 ml

EDTA (disodium salt)	18.612 g
Add sterile deionized water up to	100 ml

1% Triton X-100: 10 ml

100% Triton-X 100	100 µl
Ultrapure water (Gibco)	9.9 ml

0.1% Triton X-100: 10 ml

1% Triton-X 100	1 ml
Ultrapure water (Gibco)	9 ml

Tris wash

Stock 10X Tris wash pH 7.5: 1000 ml

Tris base	25 g
NaCl	12 g
Tween 20	2 g.
Adjust pH to 7.5	
Add sterile deionized water up to	1,000 ml

Working 1X Tris wash: 500 ml

10X Tris wash	50 ml
Sterile deionized water	450 ml

3. LB broth medium

Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Add sterile deionized water up to	1,000 ml

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