APPENDICES

APPENDIX A

Nucleic acid codes, amino acid codes,

and genetic codes

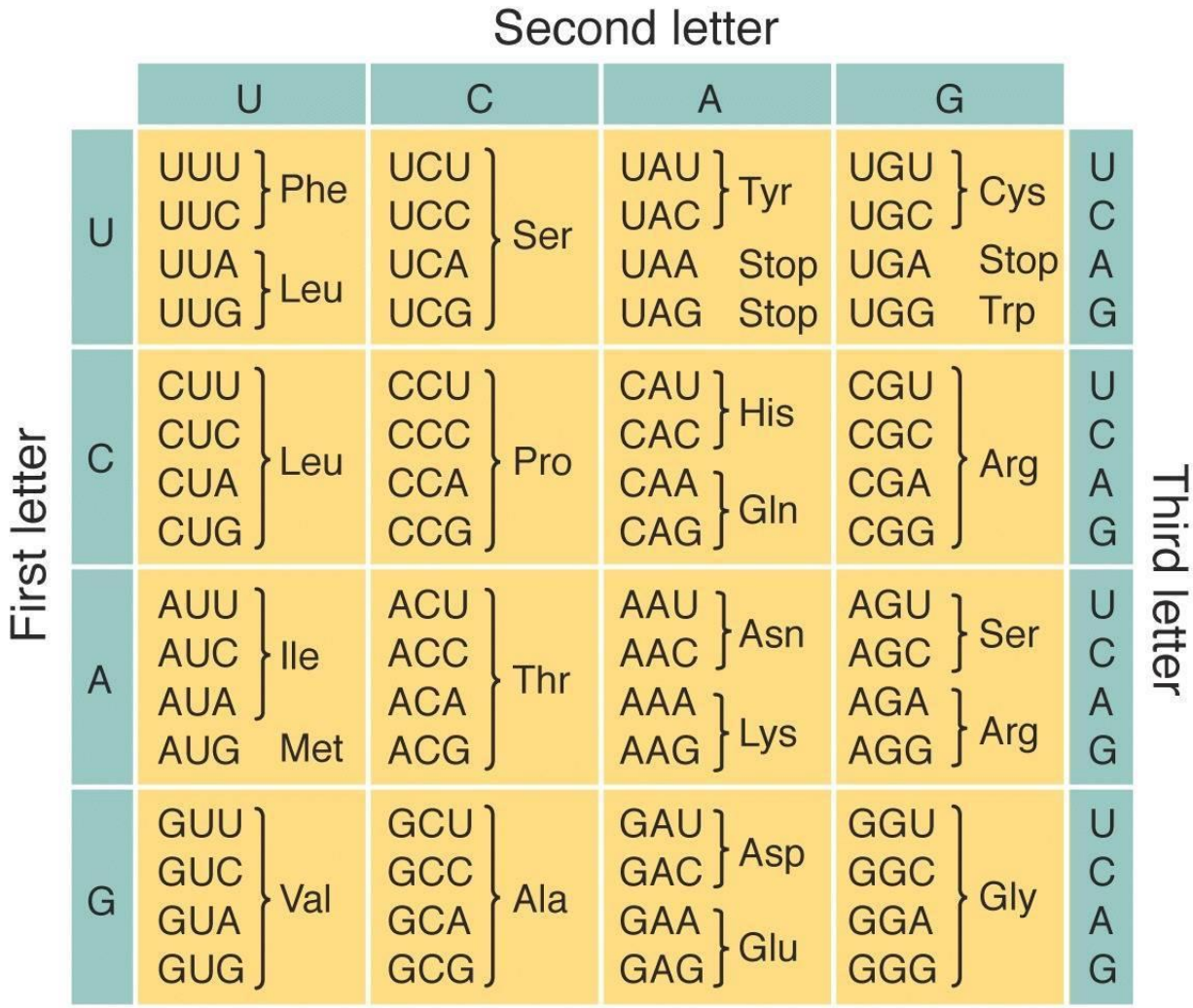
Table 6.1 Nucleic acid codes

Code	Description
A	Adenine
G	Guanine
C	Cytosine
T	Thymine
U	Uracil
R	Purine (A or G)
Y	Pyrimidine (C or T)
N	Any nucleotide
W	Weak (A or T)
S	Strong (G or C)
M	Amino (A or C)
K	Keto (G or T)
B	Not A (G or C or T)
H	Not G (A or C or T)
D	Not C (A or G or T)
V	Not T (A or G or C)

Table 6.2 Amino acid codes

1-letter code	3-letter code	Description
A	Ala	Alanine
R	Arg	Arginine
N	Asn	Asparagine
D	Asp	Aspartic acid
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine
B	Asx	Asn or Asp
Z	Glx	Gln or Glu
J	Xle	Leu or Ile
U	Sec	Selenocysteine (UGA)
O	Pyl	Pyrrolysine (UAG)
X	Unk	Unknown
Y	Tyr	Tyrosine

Figure 6.1 Standard genetic codes



(Source: http://i1.wp.com/www.jargonwall.com/wp-content/uploads/2014/12/genetic-code.jpg)

APPENDIX B

SUMMARY OF HIV-1 DRUG RESISTANCE MUTATIONS

(2015, Stanford HIV drug resistance database, USA)

Major HIV-1 Drug Resistance Mutations

Updated March 9, 2015

Updated summary from the HIV Drug Resistance Database. This document can be downloaded from the http://hivdb.stanford.edu home page. Detailed and referenced versions of each drug class summary can be found at http://hivdb.stanford.edu/pages/drugSummaries.html

Major Nucleoside RT Inhibitor (NRTI)-Resistance Mutations

	Non-TAMs					TAMs						MDR	
	184	65	70	74	115	41	67	70	210	215	219	69	151
Cons	M	K	K	L	Y	M	D	K	L	T	K	T	Q
3TC	VI	R	E									Ins	M
FTC	VI	R	E									Ins	M
ABC	VI	R	E	VI	F	L			W	YF		Ins	M
TDF	***	R	E		F	L			W	YF		Ins	M
ZDV	***	***	*	*		L	N	R	W	YF	QE	Ins	M

Bold/underline: High-level reduced susceptibility or virological response. Bold: reduced suceptibility or virological response. Plain text: reduced susceptibility in combination with other NRTI-resistance mutations. Asterisk: increased susceptibility. **Additional NRTIs:** Stavudine (d4T) and didanosine (ddI) are no longer recommended. **TAMs:** Thymidine analog mutations. Selected by AZT and d4T and facilitate primer unblocking. Non-TAMs prevent NRTI incorporation. **MDR:** Multidrug resistance mutations. T69 insertions occur with TAMs. Q151M occurs with non-TAMs and accessory mutations A62V, V75I, F77L, and F116Y. **M184VI:** Although they cause high-level *in vitro* resistance to 3TC/FTC, they are not contraindications to 3TC/FTC because they increase TDF and AZT susceptibility and decrease viral replication fitness. **Additional mutations:** K65N is similar but weaker than K65R. K70GQ is similar to K70E. T69D and V75MT reduce susceptibility to d4T and ddI. T215SCDEIV (T215 revertants) evolve from T215YF in the absence of NRTIs. E40F, E44DA, D67GE, V118I, and K219NR are accessory TAMs. T69 deletions occur in combination with K65R and/or Q151M. With K65R (but not Q151M) they increase AZT susceptibility. **References:** http://hivdb.stanford.edu/DR/NRTIResiNote.html.

Major Non-Nucleoside RT Inhibitor (NNRTI)-Resistance Mutations

	100	101	103	106	138	179	181	188	190	227	230
Cons	L	K	K	V	E	V	Y	Y	G	F	M
NVP	I	PEH	NS	AM		DEF	CIV	LCH	ASEQ	LC	L
EFV	I	PEH	NS	AM		DEF	C	LCH	ASEQ	LC	L
ETR	I	PEH					CIV		EQ	C	L
RPV	I	PEH			KAGQ	DEF	CIV	L	EQ	C	L

Bold/underline: High-level reduced susceptibility or virological response. Bold: reduced suceptibility or virological response. Plain text: reduced susceptibility in combination with other NNRTI-resistance mutations. Asterisk: increased susceptibility. **Abbreviations**: nevirapine (NVP), efavirenz (EFV), etravirine (ETR), rilpivirine (RPV). **Synergistic combinations:** V179D+K103R reduce NVP and EFV susceptibility >10-fold. Y181C+V179F cause high-level ETR and RPV resistance. **ETR genotypic susceptibility score (GSS):** Y181IV (3.0); L100I, K101P, Y181C, M230L (2.5); V90I, E138A, V179F, G190S (1.5); A98G, K101EH, V106I, V179DT, G190A (1.0); <2.5 susceptible; 2.5 to 3.0 intermediate; >3.0 high-level. V90I, A98G, V106I, E138A, V179DT, G190A/S have little effect on ETR susceptibility unless they occur with a bolded mutations. **Additional accessory mutations:** V90I (ETR), A98G (NVP, EFV, ETR, RPV), V108I, V179T (ETR), V179L (RPV), P225H (EFV), K238T (NVP, EFV), L318F (NVP). **References:** http://hivdb.stanford.edu/DR/NNRTIResiNote.html.

Major Protease Inhibitor (PI) Resistance Mutations

	24	32	46	47	48	50	54	76	82	84	88	90
Cons	L	V	M	I	G	I	I	L	V	I	N	L
ATV/r		**I**	IL	V	**VM**	**L**	**VTAM**		**ATSF**	**V**	**S**	M
DRV/r		**I**		**VA**		**V**	**LM**	**V**	**F**	V		
LPV/r	I	**I**	IL	**VA**	VM	**V**	**VTALM**	**V**	**ATSF**	**V**		M

Bold/underline: High-level reduced susceptibility or virological response. Bold: reduced suceptibility or virological response. Plain text: reduced susceptibility in combination with other PI-resistance mutations. **Abbreviations:** atazanavir (ATV), darunavir (DRV), lopinavir (LPV). Administered with ritonavir for pharmacokinetic boosting (/r). **Additional PIs:** Fosamprenavir (FPV), indinavir (IDV), saquinavir (SQV), and tipranavir (TPV) are rarely used. Nelfinavir (NFV) is no longer recommended. FPV/r and IDV/r are never more active than DRV/r and rarely if ever more active than LPV/r vs resistant viruses. TPV/r is occasionally useful for salvage therapy as it can be active vs LPV/r and DRV/r-resistant viruses with mutations that increase TPV susceptibility. Expert consultation +/- phenotypic testing should be obtained prior to using FPV, FPV/r, IDV/r, SQV/r, and TPV/r. **Additional mutations:** D30N and N88D are major NFV-resistance mutations. L10F, V11I, K20TV, L23I, K43T, F53L, Q58E, A71IL, G73STCA, T74P, N83D, and L89V are common nonpolymorphic accessory mutations. L10RY, V11L, L24F, M46V, G48ASTLQ, F53Y, I54S, V82CM, I84AC, N88TG are rare nonpolymorphic variants. **Hypersusceptibility:** I50L (each PI except ATV); L10F, L24I, I50V, I54L (TPV); L76V (ATV, SQV, TPV); I47A (SQV); N88S (FPV). **References:** http://hivdb.stanford.edu/DR/PIResiNote.html.

Major Integrase Inhibitor (INI)-Resistance Mutations

	66	92	138	140	143	147	148	155
Cons	T	E	E	G	Y	S	Q	N
RAL	A	**Q**	**KA**	**SAC**	**CRH**		**HRK**	**H**
EVG	**IAK**	**Q**	**KA**	**SAC**		**G**	**HRK**	**H**
DTG		Q	KA	SAC			**HRK**	

Bold/underline: High-level reduced susceptibility or virological response. Bold: reduced suceptibility or virological response. Plain text: reduced susceptibility in combination with other INI-resistance mutations. Asterisk: increased susceptibility. **Abbreviations**: raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG). **Additional mutations:** H51Y, L74M, T97A, S153YF, G163RK, S230R, and R263K are relatively nonpolymorphic INI-selected accessory resistance mutations. E92GV, E138T, Y143KSGA, Q148N, and N155ST are unusual variants at the positions listed above. P145S and Q146P are rare EVG-resistance mutations. G118R and F121Y are rare nonpolymorphic INI-resistance mutations. **References:** http://hivdb.stanford.edu/DR/INIResiNote.html.

HIV-1 RT and Protease Mutations for Drug-Resistance Surveillance*

NRTIs				NNRTIs		PIs			
M41	L	**Q151**	M	**L100**	I	**L23**	I	**G73**	S,T,C,A
K65	R	**M184**	V,I	**K101**	E,P	**L24**	I	**L76**	V
D67	N,G,E	**L210**	W	**K103**	N,S	**D30**	N	**V82**	A,T,S,F,L,C,M
T69	D,Ins	**T215**	Y,F,S,C,D,E,I,V	**V106**	A,M	**V32**	I	**N83**	D
K70	R,E	**K219**	Q,E,N,R	**V179**	F	**M46**	I,L	**I84**	V,A,C
L74	V,I			**Y181**	C,I,V	**I47**	V,A	**I85**	V
V75	M,T,A,S			**Y188**	L,C,H	**G48**	V,M	**N88**	D,S
F77	L			**G190**	A,S,E	**I50**	V,L	**L90**	M
Y115	F			**P225**	H	**F53**	F,Y		
F116	Y			**M230**	L	**I54**	V,L,M,T,A,S		

*Bennett DE, Camacho RJ, Otelea D,et al. Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update, PLoS One 2009;4:e4724.Criteria for mutations on this list: (i) Cause/contribute to resistance. (ii) Nonpolymorphic (≤ 0.5% in ARV-naive persons) in 8 most common group M subtypes. http://hivdb.stanford.edu/cgi-bin/MutPrevBySubtypeRx.cgi.

Table 6.3 Resistance mutation comments (Stanford HIVDR database).

NRTI mutations			
Position	**Cons**	**AA**	**Comment**
40	E	F	E40F is a nonpolymorphic mutation selected by AZT and d4T. It usually occurs in combination with M41L, L210W and T215Y. In this context it is associated with reduced susceptibility to each of the NRTIs.
41	M	I	M41I is usually an artifact resulting from APOBEC3G-mediated hypermutation.
41	M	L	M41L is a TAM that usually occurs with T215Y. Together, M41L and T215Y confer high-level resistance to AZT and d4T and intermediate-level resistance to ddI, ABC and TDF. However, viruses with M41L + T215Y + M184V will exhibit intermediate-level resistance to AZT and d4T and low-level resistance to TDF.
44	E	AD	E44A/D are minimally polymorphic accessory NRTI-resistance mutations that usually occur with multiple TAMs.
62	A	V	A62V is an accessory mutation that often occurs in combination with the multinucleoside resistance mutations K65R or Q151M-. Alone it does not reduce NRTI susceptibility. A62V is widespread in subtype A viruses in former Soviet Union countries but is otherwise nonpolymorphic.
65	K	E	K65R causes intermediate/high-level resistance to TDF, ddI, ABC and d4T (2 to 3-fold reduced susceptibility) and low to intermediate-level resistance to 3TC and FTC (5 to 7-fold reduced susceptibility). K65R increases susceptibility to AZT. K65E is an extremely rare NRTI-selected mutation with markedly reduced replication fitness.
65	K	N	K65R causes intermediate/high-level resistance to TDF, ddI, ABC and d4T (2 to 3-fold reduced susceptibility) and low to

Table 6.3 (Continued)

NRTI mutations			
Position	**Cons**	**AA**	**Comment**
			intermediate-level resistance to 3TC and FTC (5 to 7-fold reduced susceptibility). K65R increases susceptibility to AZT. K65N is a rare mutation with effects on NRTI susceptibility that are similar but weaker to those of K65R.
65	K	R	K65R causes intermediate/high-level resistance to TDF, ddI, ABC and d4T (2 to 3-fold reduced susceptibility) and low to intermediate-level resistance to 3TC and FTC (5 to 7-fold reduced susceptibility). K65R increases susceptibility to AZT.
67	D	EGS TQH	D67N is a nonpolymorphic TAM associated with low-level resistance to AZT and d4T. When present with other TAMs, it reduces susceptibility to ABC, TDF and ddI. D67G/E/S/T/H are nonpolymorphic NRTI-selected mutations that also generally occur in viruses with multiple TAMs.
67	D	N	D67N is a nonpolymorphic TAM associated with low-level resistance to AZT and d4T. When present with other TAMs, it reduces susceptibility to ABC, TDF and ddI.
67	D	delet e	Amino acid deletions (d) between codons 66 to 71 are rare and usually occur in combination with multiple TAMs, the Q151M mutation complex, or K65R. Deletions at position 67 are more often associated with multiple TAMs. Deletions at position 69 are more often associated with either the Q151M complex or K65R.
69	T	D	T69D is a nonpolymorphic mutation that reduces susceptibility to ddI and possibly d4T.
69	T	G	T69G is a rare polymorphic mutation that usually occurs in viruses with a deletion at codon 67 and multiple NRTI-resistance mutations. It is associated with reduced

Table 6.3 (Continued)

NRTI mutations			
Position	**Cons**	**AA**	**Comment**
			susceptibility to ddI, d4T, ABC and possibly TDF.
69	T	N	T69N is a relatively non-polymorphic mutation weakly selected in patients receiving NRTIs. Their effects on NRTI susceptibility have not been well studied.
69	T	SAIE	T69S/A/I/E are relatively non-polymorphic mutations weakly selected in patients receiving NRTIs. Their effects on NRTI susceptibility have not been well studied.
69	T	delete	Amino acid deletions (d) between codons 66 to 71 are rare and usually occur in combination with multiple TAMs, the Q151M mutation complex, or K65R. Deletions at position 67 are more often associated with multiple TAMs. Deletions at position 69 are more often associated with either the Q151M complex or K65R.
69	T	insert	Double amino acid insertions between codons 66 to 71 most often align to codon 69 and occur in less than 1% of heavily treated persons. Together with TAMs, they confer high-level resistance to AZT, d4T, ddI, ABC and TDF and intermediate/high-level resistance to 3TC and FTC.
70	K	EG	K70E/G cause low/intermediate-level resistance (2 to 3-fold reduced susceptibility) to TDF, ABC, DDI and possibly 3TC and FTC. K70E increases susceptibility to AZT.
70	K	QNST	K70R causes intermediate-level resistance to AZT and possibly low-level resistance to d4T and TDF. K70E/G cause low/intermediate-level resistance (2 to 3-fold reduced susceptibility) to TDF, ABC, DDI and possibly 3TC and FTC. K70E increases susceptibility to AZT. K70Q/N/S/T are rare nonpolymorphic NRTI-selected mutations that appear to have resistance profiles similar to K70E/G.
70	K	R	K70R causes intermediate-level resistance to AZT and

Table 6.3 (Continued)

NRTI mutations			
Position	**Cons**	**AA**	**Comment**
			possibly low-level resistance to d4T and TDF.
74	L	IV	L74V/I cause high-level resistance to ddI and intermediate-level resistance to ABC. L74V increases susceptibility to AZT and TDF, but this increase is of uncertain clinical significance.
75	V	I	V75I is a relatively nonpolymorphic accessory mutation that usually occurs in combination with the multi-nucleoside resistance mutations F77L, F116Y and Q151M. V75I occasionally occurs alone and in this context its clinical significance is unknown.
75	V	M	V75M appears to cause intermediate-level d4T resistance and low-level ddI resistance.
75	V	SAL	V75S/A/L are nonpolymorphic mutations that appear to reduce susceptibility to d4T and ddI.
75	V	T	V75T causes high-level d4T resistance and intermediate-level ddI resistance.
77	F	L	F77L usually occurs in combination with the multinucleoside resistance mutations F116Y and Q151M.
115	Y	F	Y115F causes intermediate-level resistance to ABC and low-level resistance to TDF.
116	F	Y	F116Y usually occurs in combination with the multinucleoside resistance mutations F77L and Q151M.
118	V	I	V118I is a polymorphic accessory NRTI-resistance mutation that occurs in combination with multiple TAMs.
151	Q	L	Q151M causes intermediate/high-level resistance to AZT, ddI, d4T and ABC and low-level resistance to TDF, 3TC and FTC. In combination with mutations at the associated positions 75, 77, and 116, Q151M confers high-level resistance to AZT, ddI, d4T and ABC and intermediate-level

Table 6.3 (Continued)

NRTI mutations			
Position	**Cons**	**AA**	**Comment**
			resistance to TDF, 3TC and FTC. Q151L is an extremely rare transitional mutation that may precede the emergence of the Q151M.
151	Q	M	Q151M causes intermediate/high-level resistance to AZT, ddI, d4T and ABC and low-level resistance to TDF, 3TC and FTC. In combination with mutations at the associated positions 62, 75, 77, and 116, Q151M confers high-level resistance to AZT, ddI, d4T and ABC and intermediate-level resistance to TDF, 3TC and FTC.
184	M	VI	M184V/I cause high-level resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication. In combination with K101E or E138K, M184I synergistically reduces RPV susceptibility.
210	L	FS	L210F/S are rare mutations not associated with NRTI-resistance.
210	L	W	L210W usually occurs in combination with M41L and T215Y. The combination of M41, L210W and T215Y causes high-level resistance to AZT and d4T and intermediate to high-level resistance to ddI, ABC and TDF.
215	T	F	T215F is a TAM that causes intermediate/high-level resistance to AZT and d4T and low-level resistance to ABC, ddI and TDF. Compared with T215Y, T215F occurs more commonly with the Type II TAMs (D67N, K70R, and/or K219E) and in this context, it affects susceptibility to TDF, ABC, and ddI less markedly than T215Y.
215	T	SCD	T215Y/F cause intermediate/high-level resistance to AZT

Table 6.3 (Continued)

NRTI mutations			
Position	**Cons**	**AA**	**Comment**
		EIV ALN	and d4T and low-level resistance to ABC, ddI and TDF. T215S/C/D/E/I/V/N/A/L do not reduce NRTI susceptibility but arise from viruses that once contained T215Y/F. The presence of one of these revertant mutations suggests the possibility that the patient may have once harbored a majority virus population with T215Y/F.
215	T	Y	T215Y is a TAM which causes intermediate/high-level resistance to AZT and d4T and low-level resistance to ABC, ddI, and TDF.
219	K	NR	K219N/R are accessory TAMS that usually occur in combination with multiple other TAMs.
219	K	QE	K219Q/E are accessory TAMS associated with reduced susceptibility to AZT and possibly d4T.
219	K	W	K219W is an uncommon NRTI-selected mutation
348	N	I	N348I is a nonpolymorphic accessory mutation selected by the NRTIs AZT and d4T and by NVP and EFV. Alone it reduces AZT and NVP susceptibility by about 3-fold and EFV susceptibility by 2-fold.
NNRTIs mutations			
90	V	I	V90I is a polymorphic accessory mutation that is weakly selected in patients by each of the NNRTIs. It has a weight of 1.0 in the Tibotec ETR genotypic susceptibility score but is associated with minimal, if any, detectable reduction in NNRTI susceptibility.
98	A	G	A98G is a nonpolymorphic accessory mutation that reduces NVP susceptibility by ~5-fold and EFV susceptibility by about 3-fold. It has a weight of 1.0 in the Tibotec ETR genotypic susceptibility score.

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
100	L	I	L100I is a nonpolymorphic mutation that usually occurs in combination with K103N. In this setting it causes high-level resistance to NVP and EFV (>50-fold reduced susceptibility), high-level resistance to RPV (>10-fold reduced susceptibility) and intermediate-level resistance to ETR (~5-fold reduced susceptibility). It has a weight of 2.5 in the Tibotec ETR genotypic susceptibility score.
100	L	V	L100V is an extremely rare nonpolymorphic mutation associated with 5 to 10-fold reduced susceptibility to NVP and EFV. It may also reduce susceptibility to ETR and RPV.
101	K	E	K101E is a nonpolymorphic mutation that causes intermediate resistance to NVP (~5-fold reduced susceptibility) and low-level resistance (~2-fold reduced susceptibility) to EFV, ETR and RPV. It has a weight of 1.0 in the Tibotec ETR genotypic susceptibility score. In combination with M184I it reduces RPV susceptibility by about 5-fold.
101	K	H	K101H is a nonpolymorphic accessory NNRTI-resistance mutation selected by NVP, EFV and ETR. When present with other NNRTI-resistance mutations, K101H further reduces susceptibility to these NNRTIs. It has a weight of 1.0 in the Tibotec ETR genotypic susceptibility score.
101	K	N	K101N/A/T are uncommon nonpolymorphic NNRTI-selected mutation of uncertain phenotypic and clinical significance.
101	K	P	K101P is a nonpolymorphic mutation that causes high-level resistance (>50-fold reduced susceptibility) to NVP, EFV and RPV and intermediate resistance (~5-fold reduced susceptibility) to ETR. It has a weight of 2.5 in the Tibotec

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
			ETR genotypic susceptibility score.
101	K	Q	K101Q is a relatively nonpolymorphic mutation that is weakly selected in patients receiving NVP and EFV. It is of uncertain phenotypic and clinical significance.
101	K	R	K101R is an uncommon polymorphism that is not associated with reduced NNRTI susceptibility.
103	K	EQ	K103E/Q are rare mutations that have not been associated with reduced susceptibility to the current NNRTIs.
103	K	H	K103H is a rare nonpolymorphic mutation that causes high-level resistance (~20-fold reduction in susceptibility) to NVP and EFV.
103	K	N	K103N is a nonpolymorphic mutation that causes high-level resistance to NVP (~50-fold reduced susceptibility) and EFV (~20-fold reduced susceptibility).
103	K	R	K103R is a polymorphic mutation that by alone has no effect on NNRTI susceptibility. However, in combination with V179D (and possibly V179E), it reduces NVP and EFV susceptibility about 15-fold.
103	K	S	K103S is a nonpolymorphic mutation that causes intermediate/high-level resistance to NVP and low/intermediate-level resistance to EFV. Because K103S is a 2-bp change from the wildtype K, patients with K103S may be more likely to harbor K103N (which is just a 1-bp change from wildtype).
103	K	T	K103T is an extremely rare nonpolymorphic mutation that appears to cause intermediate/high-level resistance to NVP (~10-fold reduction in susceptibility), but it has little if any effect on EFV susceptibility.
106	V	A	V106A is a nonpolymorphic mutation that causes high-level

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
			resistance to NVP (~50-fold reduced susceptibility) and intermediate-level resistance to EFV (~5-fold reduction in susceptibility). Together, V106A and F227L cause high-level resistance to both NVP and EFV.
106	V	I	V106I is a polymorphic NNRTI-selected accessory mutation. It has a weight of 1.5 in the Tibotec ETR genotypic susceptibility score. It has minimal, if any, effect on NNRTI susceptibility.
106	V	M	V106M is a nonpolymorphic mutation that causes high-level resistance (>30-fold reduced susceptibility) to NVP and EFV.
108	V	I	V108I is a relatively nonpolymorphic accessory mutation selected in patients receiving NVP, EFV and ETR. It causes low-level resistance (~2-fold reduction in susceptibility) to NVP and EFV. It does not appear to reduce susceptibility to ETR or RPV.
138	E	A	E138A is a common polymorphic accessory mutation weakly selected in patients receiving ETR and RPV. It reduces ETR and RPV susceptibility ~2-fold. It has a weight of 1.5 in the Tibotec ETR genotypic susceptibility score.
138	E	GQ	E138Q/G are nonpolymorphic accessory mutations frequently selected in patients receiving ETR and RPV and occasionally in patients receiving NVP and EFV. E138Q/G are associated with 2 to 3-fold reduced susceptibility to ETR and RPV.
138	E	K	E138K is a nonpolymorphic mutation selected in a high proportion of patients receiving RPV. Alone it causes low-level RPV resistance (2 to 3-fold reduced susceptibility). However, in combination with the NRTI-resistance

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
			mutation M184I it appears sufficient to cause virological failure on an RPV-containing regimen. E138K causes low-level cross-resistance to ETR but minimal, if any, cross-resistance to NVP and EFV.
138	E	R	E138R is a rare nonpolymorphic accessory mutation selected in vitro by RPV. It is associated with 2 to 3-fold reduced susceptibility to ETR and RPV.
179	V	DE	V179D is a polymorphic accessory mutation selected in patients receiving EFV. It reduces NVP and EFV susceptibility by 2 to 5-fold and ETR and RPV susceptibility ~2-fold. The combination of V179D and K103R act synergistically to reduce NVP and EFV susceptibility >10-fold. V179D has a weight of 1.0 in the Tibotec ETR GSS. V179E is a nonpolymorphic mutation infrequently selected by NVP and EFV. V179E appears to be similar to V179D in its effects on NNRTIs.
179	V	F	V179F is a nonpolymorphic mutation frequently selected in patients receiving ETR. It nearly always occurs in combination with Y181C. Alone V179F has little effect on NNRTI susceptibility. In combination with Y181C, however, it is associated with high-level ETR and RPV resistance (>10-fold reduced susceptibility). It has a weight of 1.5 in the Tibotec ETR GSS.
179	V	I	V179I is a polymorphic mutation that is frequently selected in patients receiving ETR and RPV. It has little, if any, effect on NNRTI susceptibility.
179	V	L	V179L is a rare nonpolymorphic mutation infrequently selected in patients receiving NVP, EFV and RPV. Its effects on NNRTI susceptibility have not been well studied.

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
			It is listed as an RPV-associated drug-resistance mutation in the RPV package insert.
179	V	T	V179T is a rare nonpolymorphic mutation infrequently selected in patients receiving NNRTIs. It is associated with minimal reductions in ETR and RPV susceptibility. It has a weight of 1.0 in the Tibotec ETR GSS.
181	Y	C	Y181C is a nonpolymorphic mutation selected in patients receiving NVP, ETR and RPV. It reduces susceptibility to NVP, ETR, RPV, and EFV by >50-fold, 5-fold, 3-fold, and 2-fold, respectively. Although Y181C itself reduces EFV susceptibility by only 2-fold, it is associated with a reduced response to an EFV-containing regimen because viruses with this mutation often harbor additional minority variant NNRTI-resistance mutations. Y181C has a weight of 2.5 in the Tibotec ETR GSS.
181	Y	FSG	Y181F/S/G are rare nonpolymorphic NNRTI-associated mutations that are usually present as part of an electrophoretic mixture. They are likely to represent transitional mutations between Y and I or V.
181	Y	IV	Y181I/V are 2-base pair nonpolymorphic mutations selected by NVP and ETR. Y181I/V cause high-level resistance to NVP (>50-fold reduced susceptibility) and to ETR and RVP (10 to 15-fold reduced susceptibility). Y181I/V each have a weight of 3.0 in the Tibotec ETR genotypic susceptibility score.
188	Y	C	Y188C is a nonpolymorphic mutation selected in patients receiving NVP and EFV. It confers high-level resistance to NVP (>50-fold reduced susceptibility) and EFV (~20-fold reduced susceptibility).

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
188	Y	F	Y188F is a rare nonpolymorphic NNRTI-associated mutations that is usually present as part of an electrophoretic mixture. It appears to represent a transitional mutation between Y and L.
188	Y	H	Y188H is a nonpolymorphic mutation selected in patients receiving NVP and EFV. It causes about 5 to 10-fold reduced susceptibility to NVP and EFV.
188	Y	L	Y188L is a nonpolymorphic mutation that causes high-level resistance (>50-fold reduced susceptibility) to NVP and EFV and intermediate/high-level resistance (5-fold reduced susceptibility) to RPV.
190	G	A	G190A is a nonpolymorphic mutation that causes high-level resistance to NVP (>50-fold reduced susceptibility) and intermediate resistance to EFV (5 to 10-fold reduced susceptibility). It has a weight of 1.0 in the Tibotec ETR GSS but does not appear to be selected by ETR or RPV or to reduce their in vitro susceptibility.
190	G	CTV	G190C/T/V are rare nonpolymorphic mutations that cause high-level resistance to NVP and EFV (>50-fold reduced susceptibility). Their effects on ETR and RPV susceptibility are not known.
190	G	EQ	G190E/Q are nonpolymorphic mutations that cause high-level resistance to NVP and EFV (>50-fold reduced susceptibility). Both mutations also appear to be associated with high-level resistance (>10-fold reduced susceptibility) to RPV and ETR.
190	G	S	G190S is a nonpolymorphic mutation that causes high-level resistance to NVP and EFV (>50-fold reduced susceptibility). It has a weight of 1.5 in the Tibotec ETR

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
			genotypic susceptibility score but does not appear to be selected by ETR or RPV.
221	H	Y	H221Y is a nonpolymorphic accessory NNRTI-selected mutation that frequently occurs in combination with Y181C. Alone it has minimal detectable effects on NNRTI susceptibility. It is frequently selected in patients receiving RPV (Rimsky 2012, Tibotec 2012).
225	P	H	P225H is a nonpolymorphic EFV-selected mutation that usually occurs in combination with K103N. The combination of P225H and K103N causes a >50-fold reduction in EFV susceptibility.
227	F	C	F227C is an extremely rare nonpolymorphic mutation selected in patients receiving RPV and in vitro by ETR and RPV. It usually occurs in combination with other NNRTI-resistance mutations and in this context it is associated with high-level resistance to each of the NNRTIs.
227	F	L	F227L is a nonpolymorphic mutation that usually occurs in combination with V106A. In this setting it is associated with high-level resistance to NVP and EFV.
230	M	I	M230I is an extremely rare mutation selected in vitro by RPV. Its effects on NNRTI susceptibility have not been well studied.
230	M	L	M230L is an uncommon nonpolymorphic mutation selected in patients receiving EFV, NVP, and RPV. It causes intermediate to high-level resistance to each of the NNRTIs.
236	P	L	P236L is a nonpolymorphic mutation that causes high-level DLV resistance but does not reduce susceptibility to any other NNRTIs.
238	K	R	K238R is a common polymorphism that does not reduce

Table 6.3 (Continued)

NNRTI mutations			
Position	**Cons**	**AA**	**Position**
			NNRTI susceptibility.
238	K	TN	K238T is a nonpolymorphic mutation selected in patients receiving NVP and EFV. It usually occurs in combination with K103N. It reduces susceptibility to NVP and EFV by about 5-fold. It may also reduce susceptibility to ETR and RPV. K238N is a nonpolymorphic accessory mutation that is also selected by NVP and EFV. It appears to have minimal, if any, effects on NNRTI susceptibility.
318	Y	F	Y318F is an uncommon mutation that causes intermediate-level NVP resistance and potentially low-level EFV resistance.
348	N	I	N348I is a nonpolymorphic accessory mutation selected by the NRTIs AZT and d4T and by NVP and EFV. Alone it reduces AZT and NVP susceptibility by about 3-fold and EFV susceptibility by 2-fold.
PI Mutations			
10	L	F	L10F is a common nonpolymorphic, PI-selected accessory mutation associated with reduced susceptibility to each of the PIs except ATV, SQV, and TPV.
10	L	IV	L10I/V are polymorphic, PI-selected accessory mutations that reduce PI susceptibility or increase the replication of viruses with other PI-resistance mutations.
10	L	RY	L10R/Y are rare, nonpolymorphic PI-selected accessory mutations. Their effects on PI susceptibility have not been well studied.
11	V	IL	V11I is a minimally polymorphic PI-resistance accessory mutation that is often selected in patients receiving DRV. It is associated with minimal reductions in DRV and FPV

Table 6.3 (Continued)

PI mutations			
Position	**Cons**	**AA**	**Position**
			susceptibility. It is included in the Tibotec GSS for DRV. V11L is a nonpolymorphic accessory PI-resistance mutation that is also associated with minimal reductions in DRV and FPV susceptibility.
20	K	I	K20I is the consensus amino acid in subtype G and CRF02_AG. In subtypes B and C, K20I is a PI-selected mutation that appears to reduce NFV susceptibility.
20	K	MV	K20M/V are rare, relatively nonpolymorphic PI-selected mutations that have not been well studied.
20	K	R	K20R is a highly polymorphic, PI-selected accessory mutation that improves HIV-1 replication fitness in viruses with other PI-resistance mutations.
20	K	T	K20T is a nonpolymorphic accessory PI-selected mutation that is associated with reduced susceptibility to each of the PIs except SQV and TPV.
23	L	I	L23I is an uncommon nonpolymorphic mutation selected primarily by NFV. It causes low/intermediate-level resistance to NFV.
24	L	FM	L24F is an uncommon nonpolymorphic PI-selected mutation that appears to have a susceptibility profile similar to L24I. L24M is a rare nonpolymorphic PI-selected mutation that has not been well studied.
24	L	I	L24I is a nonpolymorphic mutation selected by IDV and, less often, LPV. It reduces susceptibility to FPV, IDV, LPV, SQV, ATV and NFV. It increases susceptibility to TPV.
30	D	N	D30N is a nonpolymorphic substrate-cleft mutation that causes high-level resistance to NFV.
32	V	I	V32I is a nonpolymorphic substrate-cleft mutation associated with reduced susceptibility to each PI except

Table 6.3 (Continued)

PI mutations			
Position	**Cons**	**AA**	**Position**
			SQV. It is included in the Tibotec DRV GSS.
33	L	F	L33F is a relatively nonpolymorphic accessory mutation selected by DRV, FPV, LPV, NFV and TPV. In combination with other PI-resistance mutations, L33F is associated with reduced susceptibility to these PIs. It is included in the Tibotec DRV GSS.
33	L	I	L33I is a relatively nonpolymorphic PI-selected mutation that appears to have minimal, if any, effects on PI susceptibility.
33	L	V	L33V is a polymorphism that does not appear to be selected by PIs or to be associated with reduced PI susceptibility.
35	E	G	E35G is a relatively nonpolymorphic PI-selected mutation that is weakly associated with reduced NFV and TPV susceptibility.
43	K	T	K43T is a nonpolymorphic PI-selected accessory mutation that, in combination with other PI-resistance mutations, is associated with reduced susceptibility to most PIs. It is also part of the GSS for TPV.
46	M	IL	M46I/L are nonpolymorphic PI-selected mutations that reduce susceptibility to IDV, NFV, FPV, LPV and ATV when present with other mutations. M46L also reduces susceptibility to TPV.
46	M	V	M46I/L are nonpolymorphic PI-selected mutations that reduce susceptibility to IDV, NFV, FPV, LPV and ATV when present with other mutations. M46L also reduces susceptibility to TPV. M46V is a rare nonpolymorphic PI-selected mutation that has not been well studied.
47	I	A	I47A is a nonpolymorphic mutation selected by LPV. It usually occurs in combination with V32I and it confers

Table 6.3 (Continued)

PI mutations			
Position	**Cons**	**AA**	**Position**
			high-level resistance to LPV and FPV and low/intermediate-resistance to the remaining PIs except ATV and SQV. It increases susceptibility to SQV.
47	I	V	I47V is a nonpolymorphic mutation selected by IDV, FPV, LPV and DRV. It is associated with reduced susceptibility to each of the PIs except SQV and ATV. I47V is included in the Tibotec DRV GSS.
48	G	AST QL	G48V causes high-level resistance to SQV, intermediate-level resistance to ATV and NFV, and low-level resistance to IDV and LPV. G48M is a less common mutation that appears to have similar effects on PI susceptibility. G48A/S/T/Q are rare nonpolymorphic PI-selected mutations that occur in patients who have received multiple PIs.
48	G	M	G48V is a nonpolymorphic substrate-cleft mutation selected by SQV and, less often, by IDV and LPV. It confers high-level resistance to SQV, intermediate-level resistance to ATV, and low-level resistance to NFV, IDV and LPV. G48M is a 2-base pair nonpolymorphic mutation selected in patients who have received multiple PIs. It causes high-level resistance to SQV, intermediate-level resistance to ATV and NFV and low-level resistance to IDV and LPV.
48	G	V	G48V is a nonpolymorphic substrate-cleft mutation selected by SQV and, less often, by IDV and LPV. It confers high-level resistance to SQV, intermediate-level resistance to ATV, and low-level resistance to NFV, IDV and LPV.
50	I	L	I50L is a nonpolymorphic substrate-cleft mutation selected by ATV. It causes intermediate/high-level resistance to ATV and increases susceptibility to the remaining PIs.
50	I	V	I50V is a nonpolymorphic substrate-cleft mutation selected

Table 6.3 (Continued)

PI mutations			
Position	**Cons**	**AA**	**Position**
			by FPV, LPV and DRV. It reduces susceptibility to these PIs and increases susceptibility to TPVI50V is included in the Tibotec DRV GSS.
53	F	LY	F53L is a nonpolymorphic mutation selected primarily by SQV, IDV, ATV, and LPV. It reduces susceptibility primarily to ATV, SQV, and NFV. F53Y is a rare nonpolymorphic PI-selected mutation that has not been well studied.
54	I	L	I54L is a nonpolymorphic mutation selected by FPV, LPV and DRV. It reduces susceptibility to these PIs and possibly to ATV, IDV, NFV and SQV. It increases susceptibility to TPV. It is in the Tibotec DRV GSS.
54	I	M	I54M is a nonpolymorphic mutation selected by FPV, LPV and DRV. It reduces susceptibility to each of the PIs. It is in the Tibotec DRV GSS.
54	I	TAS	I54A/T/S are nonpolymorphic PI-selected mutations that occur almost exclusively in patients who have received multiple PIs. I54A/T/S are associated with reduced susceptibility to each of the PIs except DRV.
54	I	V	I54V is a nonpolymorphic mutation selected primarily by IDV and LPV. It reduces susceptibility to each of the PIs except DRV. It synergistically reduces PI susceptibility when present in combination with V82 mutations.
58	Q	E	Q58E is a nonpolymorphic accessory PI-selected mutation associated with reduced susceptibility to TPV and possibly other PIs.
71	A	TVI L	A71T/V are polymorphisms that occur in 2-3% of untreated persons. They increase in prevalence in persons receiving PIs. A71I/L are nonpolymorphic mutations that occur in

Table 6.3 (Continued)

PI mutations			
Position	**Cons**	**AA**	**Position**
			viruses with multiple PI-resistance mutations.
73	G	STCA	G73S/T/C/A are nonpolymorphic mutations that are selected primarily by ATV, IDV, NFV and SQV. They are associated with reduced susceptibility to these PIs and possibly to DRV, FPV and LPV.
73	G	V	G73V is a nonpolymorphic PI-selected mutation that has not been well studied.
74	T	P	T74P is a nonpolymorphic PI-selected accessory mutation that occurs primarily in viruses from patients who have received multiple PIs. It is associated with reduced susceptibility to each of the PIs. It is included in the Boehringer-Ingelheim TPV and Tibotec DRV GSS.
74	T	S	T74S is a polymorphic mutation weakly selected by most PIs and associated with low-level resistance to NFV.
76	L	V	L76V is a nonpolymorphic mutation selected by IDV, LPV and DRV. It reduces susceptibility to these PIs and to FPV. It increases susceptibility to ATV, SQV and TPV. L76V is included in the Tibotec DRV GSS.
82	V	A	V82A is a nonpolymorphic substrate-cleft mutation selected primarily by IDV and LPV. It reduces susceptibility to these PIs and causes cross-resistance to ATV and NFV. When it occurs in combination with additional PI-resistance mutations it is also associated with reduced susceptibility to SQV and FPV.
82	V	C	V82C is an uncommon nonpolymorphic 2-base-pair substrate-cleft mutation that develops in viruses with multiple PI-resistance mutations from patients who received multiple PIs. Its effects on PI susceptibility have not been well studied.

Table 6.3 (Continued)

PI mutations			
Position	**Cons**	**AA**	**Position**
82	V	F	V82F is a nonpolymorphic substrate-cleft mutation selected primarily in patients who have received multiple PIs. It causes reduced susceptibility to DRV, FPV, IDV, LPV and NFV.
82	V	I	V82I is a highly polymorphic substrate-cleft mutation that is not selected by PIs. It is the consensus amino acid in subtype G viruses.
82	V	L	V82L is an uncommon nonpolymorphic substrate-cleft mutation selected by TPV. It reduces TPV susceptibility but its effects on other PIs have not been well studied.
82	V	M	In most subtypes, V82M is a 2-base-pair substrate-cleft mutation that develops in viruses with multiple PI-resistance mutations from patients who received multiple PIs. In subtype G, V82M is a 1-base-pair mutation. V82M reduces susceptibility to IDV, LPV and possibly other PIs.
82	V	TS	V82T is a nonpolymorphic substrate-cleft mutation selected in patients who received IDV, TPV, or multiple PIs. It reduces susceptibility to these PIs and to ATV, LPV, and NFV. V82T is included in the Boehringer-Ingelheim TPV GSS. V82S is a nonpolymorphic mutation that appears to have a resistance profile similar to V82T.
83	N	D	N83D is a nonpolymorphic mutation selected primarily in patients who have received multiple PIs. It is associated with reduced susceptibility to ATV, IDV, NFV, SQV and TPV. It is included in the Boehringer-Ingelheim GSS for TPV.
84	I	AC	I84A is a rare nonpolymorphic PI-selected substrate-cleft mutation associated with high-level resistance to each of the PIs. I84C is a rare nonpolymorphic PI-selected mutation

Table 6.3 (Continued)

PI mutations			
Position	**Cons**	**AA**	**Position**
			associated with varying degrees of reduced susceptibility to each of the PIs.
84	I	V	I84V is a nonpolymorphic substrate-cleft mutation selected by each of the PIs. It causes high-level resistance to ATV, FPV, IDV, NFV and SQV, intermediate-level resistance to LPV and TPV, and low-level resistance to DRV.
85	I	V	I85V is a nonpolymorphic PI-selected mutation. It has minimal, if any, effects on PI susceptibility.
88	N	D	N88D is selected by NFV. In combination with D30N, it synergistically reduces susceptibility to NFV. It may also be associated with reduced susceptibility to ATV and SQV.
88	N	S	N88S is a nonpolymorphic mutation selected by NFV and ATV. It causes high-level resistance to NFV and ATV and low-level resistance to IDV and SQV. It increases susceptibility to FPV.
88	N	TG	N88G/T are extremely rare nonpolymorphic PI-selected mutations that reduce susceptibility to NFV and ATV.
89	L	IT	L89T/I are nonpolymorphic PI-selected mutation of uncertain phenotypic and clinical significance.
89	L	M	L89M is a common polymorphism that is not associated with reduced PI susceptibility. It is the consensus amino acid in most non-B subtypes.
89	L	V	L89V is a nonpolymorphic accessory mutation selected by IDV, NFV, FPV, LPV and DRV. It reduces susceptibility to these PIs. L89V is included in the Tibotec DRV GSS (de Meyer 2008).
90	L	M	L90M is a nonpolymorphic mutation selected primarily by SQV, NFV, IDV and LPV. It reduces susceptibility to each of the PIs except TPV and DRV.

APPENDIX C

LIST OF THE CHEMICALS AND REAGENTS

Chemical and Reagents	Source
100 bp DNA ladder	Thermo Scientific, USA
6X gel loading ladder	Thermo Scientific, USA
Absolute ethyl alcohol	Merck, Germany
Agarose	Seakem, BMA, ME, USA
BigDye®Terminator V3.1 Cycle Sequencing Kit	Applied Biosystems, USA
Ethidium bromide	Promega, USA
Isopropanol	Merck, Germany
Nucleospin®Tissue Kit	Macherey-Nagel, Germany
Nucleospin®Gel and PCR Clean-up Kit	Macherey-Nagel, Germany
Platinum® *Taq* DNA Polymerase High Fidelity	Invitrogen, USA

APPENDIX D

LIST OF THE INSTRUMENTS

Chemical and Reagents	Source
ABI Prism 3100/3130 Genetic Analyzer	Applied Biosystems, USA
Analytical balance	Ohaus, Germany
Autoclave	Consolidated Sterilizer Systems, USA
Biological safty cabinet class I	Ibis Biosciences, USA
Dry bath incubator	Sheldon, USA
Eon™ Microplate Spectrometer	BioTek Instruments, USA
Freezer (-20°C)	Sanyo, Thailand
G:BOX F3 Gel imaging instrument	Syngene, USA
Gel electrophoresis system	Bio-rad, USA
GeneAmp®PCR System 9700	Applied Biosystems, USA
Geneious software	Biomatters, NZ
Hot air over	Sheldon, USA
Low speed centrifuge 2500 rpm	Harikul, Thailand
Microcentrifuge	Denville scientific, USA
Plate centrifuge	Eppendorf, Germany
Refrigerator	Sanyo, Thailand
Ultraviolet transilluminator	VilberLourmat, France
Vortex mixer	Daihan Scientific, Korea
Water bath	Sheldon, USA

APPENDIX G

REAGENT PREPARATIONS

1.50X TAE buffer

Tris base

Boric acid

0.5 M EDTA

- Dissolve in 100 ml distilled water

- Sterilize by autoclaving and store at room temperature

2. 10 mg/ml Ethidium bromide

Ethidium bromide	1g

- Dissolve in 100 ml distilled water
- Stir on magnetic stirrer
- Wrap the container with aluminum foil and store at 4°C in refrigerator

3. 1% agarose gel

Agarose	1g
0.5X TAE buffer	100ml

- Melt in microwave oven for 3 minutes

4. 75% Isopropanol

Isopropanol	3ml
Distilled water	1ml

CURRICULUM VITAE

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Publications

Girdthep, N., Samleerat, T. HIV Drug Resistance in Newly HIV-Infected Infants in Thailand. International Graduate Research Conference 2015. Chiang Mai, Thailand. December 11, 2015. (Proceeding).

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