

**ANRS AC11 Resistance Study Group
PCR and Sequencing Procedures: HIV-1
Version January 2015**

PCR and sequencing Primers

• **Protease**

Outer primers:

5'PROT1: 5'-TAA TTT TTT AGG GAA GAT CTG GCC TTC C-3'

3'PROT1: 5'-GCA AAT ACT GGA GTA TTG TAT GGA TTT TCA GG-3'

Inner and sequencing primers:

5'PROT2: 5'- TCA GAG CAG ACC AGA GCC AAC AGC CCC A-3'

3'PROT2: 5'- AAT GCT TTT ATT TTT TCT TCT GTC AAT GGC-3'

• **Reverse Transcriptase**

Outer primers:

MJ3 : 5'-AGT AGG ACC TAC ACC TGT CA-3'

MJ4: 5'-CTG TTA GTG CTT TGG TTC CTC T-3'

Inner and sequencing primers:

A35: 5'-TTG GTT GCA CTT TAA ATT TTC CCA TTA GTC CTA TT-3'

NE135: 5'-CCT ACT AAC TTC TGT ATG TCA TTG ACA GTC CAG CT-3'

Other sets of primers that can be alternatively used for Reverse Transcriptase:

Outer primers:

RT18: 5'-GGA AAC CAA AAA TGA TAG GGG GAA TTG GAG G -3'

RT21: 5'- CTG TAT TTC TGC TAT TAA GTC TTT TGA TGG G -3'

Inner and sequencing primers:

RT1: 5'- CCA AAA GTT AAA CAA TGG CCA TTG ACA GA -3'

RT4: 5'- AGT TCA TAA CCC ATC CAA AG -3'

Outer primers:

RT18 : 5'-GGA AAC CAA AAA TGA TAG GGG GAA TTG GAG G -3'

JPH4M: 5'-TGA ATG ATT CCT AAT GCA TAY TGT GAG TCT GT -3'

Inner and sequencing primers:

RT1: 5'- CCA AAA GTT AAA CAA TGG CCA TTG ACA GA -3'

PL1M: 5'-CYT GYT TCT GTA TTT CTG CTA YTA AGT CTT TTG -3'

- **Integrase**

Outer primers:

INPS1 : 5' - TAG TAG CCA GCT GTG ATA AAT GTC- 3'

INPR8: 5'-TTC CAT GTT CTA ATC CTC ATC CTG-3'

Inner and sequencing primers:

INPS3 : 5' - GAA GCC ATG CAT GGA CAA G- 3'

INPR9: 5'-ATC CTC ATC CTG TCT ACT TGC C -3'

Other sets of primers that can be alternatively used for Integrase

Outer primers:

HP4149 5'-CAT GGG TAC CAG CAC ACA AAG GAA TT- 3'

IN-PCRBD 5' -GCT YTC TTT GAA AYA TAC ATA TGR T- 3'

Inner and sequencing primers:

IN-PCRA 5'-GGA GGA AAT GAA CAA GTA GAT- 3'

IN-SEQ2 5'-TAC TGC TGT CTT AAG RTG TTC AG- 3'

- **Enveloppe:**

gp 120 (C2V3)

Outer primers:

Env 31: 5'-CAG TAC AAT GTA CAC ATG G-3' (6955 to 6973)

Env 8: 5'-ATG GGA GGG GCA TAC ATT G-3' (7522 to 7540).

Inner and sequencing primers:

Env 7: 5'-AAT GGC AGT CTA GCA GAA G-3' (7008 to 7026)

ED33: 5'-TTA CAG TAG AAA AAT TCC CCT C-3' (7360 to 7381)

PCR runs on 9700 PE thermocycler (times have to be adapted to the thermocycler and taq polymerase used)

Conditions for Reverse Transcriptase, Protease and Integrase (protocol 1):

- RT-PCR Step, using RT-PCR kit Titan (Roche):

30' at 50°C

5' at 94°C

then: (30'' at 94°C; 30'' at 55°C; 1' at 68°C) x 45 cycles

- Nested PCR step, using Ampli Taq gold with Gene Amp (Roche):

3' at 94°C

then (30'' at 94°C; 30'' at 55°C; 1'30'' at 72°C) x 45 cycles

7' at 72°C

Conditions for gp120 (protocol 2):

- RT-PCR Step, using RT-PCR kit Titan (Roche):

30' at 50°C

5' at 94°C

then: (30'' at 94°C; 30'' at 50°C; 1' at 68°C) x 45 cycles

- Nested PCR step, using Ampli Taq gold with Gene Amp (Roche):

3' at 94°C

then (30'' at 94°C; 30'' at 50°C; 1'30'' at 72°C) x 45 cycles

7' at 72°C