

ANRS AC43 HIV Resistance Study Group

PCR and Sequencing Procedures: HIV-1 Version 2022.1

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 set of primers which can be alternatively used for RT

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 set of primers which can be alternatively used for INT

Outer primers

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

IN-PCRB D 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)

Env (full length)

Outer primers

ExtM5: 5'-ATG GCA GGA AGA AGC GGA RRC-3'

ExtM3: 5'-CTT RTA AGT CAT TGG TCT TAA AGG YAC-3'

Inner primers

IntM5: 5'-AGA AAG AGC AGA AGA CAG TGG CAA TG-3'

IntM3: 5'-CTA CTT TTT GAC CAC TTG CCM CCC AT-3'

Sequencing primers

IntM5 (6202): 5'-AGA AAG AGC AGA AGA CAG TGG CAA TG-3'

Env 1F (6342): 5'-TAT GGR GTR CCN GTR TGG-3'

Env 1R (6342): 5'-CCA YAC NGG YAC YCC ATA-3'

Env 2F (6861): 5'-ATT CCH ATA CAY TWY TGT RC-3'

Env 2R (6861): 5'-GYA CAR WAR TGT ATD GGA AT-3'

Env 3F (7521): 5'-GCA ATR TAT GCY SCT CC-3'

Env 3R (7521): 5'-GGA GSR GCA TAY ATT GC-3'

Env 4F (7798): 5'-CAG CHG GAA GCA CKA TGG G-3'

Env 4R (7798): 5'-CCC ATM GTG CTT CCD GCT G-3'

Env 5F (8526): 5'-TAC CRC CRM TTG ASR GAC T-3'

Env 5R (8526): 5'-AGT CYS TCA AKY GGY GGT A-3'

IntM3 (8797): 5'-CTA CTT TTT GAC CAC TTG CCM CCC AT-3'

• Capsid

Outer primers

6-CA-48F : 5'-MTAGTATGGGCAAGCAGGGA-3'

7-CA-228F : 5'-RTAARAGACACCAARGAAGC-3'

Inner and sequencing primers

8-CA-998R : 5'-RCATGCTGTCATCATTCTT-3'

9-CA-1141R : 5'-TCTTTGCCACARTTGAARCA-3'

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

Conditions for RT, prot and Integrase (protocol 1) and gp120 (*protocol 2*)

- RT-PCR Step, using RT-PCR kit Access (PROMEGA):

45' at 45°C

2' at 95°C

then: (30'' at 95°C; 30'' at 50°C; 1' at 68°C) x 40 cycles

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

5' at 94°C

then (30'' at 94°C; 30'' at 55°C (50°C); 1' at 72°C) x 45 cycles

7' at 72°C

Conditions for Env (full length)

- RT Step, using Superscript III First strand synthesis (Invitrogen)

5' at 65°C

1' on ice

60' at 50°C

15' at 70°C

- PCR and Nested PCR steps, using Platinum PCR supermix high fidelity (Invitrogen)

2' at 94°C

then: (15'' at 94°C; 30'' at 55°C; 3' at 68°C) x 35 cycles

10' at 68°C