

ANRS AC11 Resistance Study Group

PCR and Sequencing Procedures: HIV-1 Version February 2008

PCR and sequencing Primers

- Reverse Transcriptase (codons 20-240):

Outer primers

MJ3: 5'-AGTAGGACCTACACCTGTCA-3' (2480 to 2499)

MJ4: 5'-CTGTTAGTGCTTTGGTTCCTCT-3' (3399 to 3420)

Inner primers (amplification: 798 bp fragment)

A(35): 5'-TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT-3' (2530 to 2558)

NE1(35): 5'-CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT-3' (3300 to 3334)

Sequencing primers

A(20): 5'-ATTTTCCCATTAGTCCTATT-3'

Or (alternatively to A(20)): HIV55 : 5' CCAAAGTTAAACAATGGCCATTGACAGA 3'

NE1(20): 5'-ATGTCATTGACAGTCCAGCT-3'

Other set of primers which can be alternatively used for RT (codons 30- 225):

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'

- Protease:

Outer primers:

5' prot 1: 5'-TAATTTTTTTAGGGAAGATCTGGCCTTCC-3' (2082 to 2109)

3' prot 1 5'-GCAAATACTGGAGTATTGTATGGATTTTCAGG-3' (2703 to 2734);

Inner (amplification: 507 bp fragment) and sequencing primers

5' prot 2: 5'-TCAGAGCAGACCAGAGCCAACAGCCCCA-3' (2136 to 2163)

3' prot 2: 5'-AATGCTTTTATTTTTTCTTCTGTCAATGGC-3' (2621 to 2650);

Other sets of primers which can be used alternatively for protease:

Outer primers:

5' eprB: 5' AGA GCT TCA GGT TTG GGG 3'

3' eprB: 5' GCC ATC CAT TCC TGG CTT 3'

Inner and sequencing primers:

5' prB: 5' GAA GCA GGA GCC GAT AGA CA 3'

3' prB: 3' ACT GGT ACA GTT TCA ATA GG 3'

- **Enveloppe:**

gp 120 (C2V3)

Protocol 1

Outer primers:

Env 31: 5'-CAGTACAATGTACACATGG-3' (6955 to 6973)

Env 8: 5'-ATGGGAGGGGCATACATTG-3' (7522 to 7540).

Inner and sequencing primers:

Env 7: 5'-AATGGCAGTCTAGCAGAAG-3' (7008 to 7026)

ED33: 5'-TTACAGTAGAAAAATCCCCTC-3' (7360 to 7381)

Or alternatively, protocol 2

Outer primers:

ED3: 5'-TAGGCATCTCCTATGGCAGGAAGAAGCGG-3' (5958 to 5986)

3'OUTENV :5'-AGTGGTGCAAATGAGTTTTCC-3' (8022 to 8042)

Inner and sequencing primers:

E5: 5'-ATCCCACATATTATTGTGCCCC-3' (6861 to 6883)

V3 Ext2 :5'-ATTACAGTAGAAAAATCCC-3'(7362-7382)

gp41 (HR1- HR2, codons 6-172)

Outer primers :

Env 1 : 5' tgg agg agg aga tat gag gg 3' (7634 to 7654)

GP2: 5' ata atg gtg aat atc cct gcc taa ctc 3' (8343 to 8370)

Inner and sequencing primers :

Env 3 : 5' ttc ctt ggg ttc ttg gga gc 3' (7779 to 7798)

Env 2 : 5' cta cca agc ctc cta cta tc 3' (8285 to 8304)

gp41 : HR1 only

Outer primers:

Env 1 : 5' tgg agg agg aga tat gag gg 3'

Env 2 : 5' cta cca agc ctc cta cta tc 3'

Inner and sequencing primers:

5' IN: 5' ttc ctt ggg ttc ttg gga gc 3'

3'OUT: 5' agt ggt gca aat gag ttt tcc 3'

Integrase

Protocol 1

Outer primers:

IN12: 5' GCAGGATTCGGGATTAGAAG 3' (4007 to 4026)

IN13: 5' CTTTCTCCTGTATGCAGACC 3' (5251 to 5270)

Inner primers:

IN1: 5' AAGGTCTATCTGGCATGGGTA 3' (4137 to 4157)

BH4: 5' TCCCCTAGTGGGATGTGTACTTC 3' (5200 to 5222)

Sequencing primers:

IN1: 5' AAGGTCTATCTGGCATGGGTA 3' (4137 to 4157)

IN4764AS: 5' CCATTTGTACTGCTGTCTTAA 3' (4744 to 4764)

IN4542S: 5' GCAGGAAGATGGCCAGT 3' (4542 to 4558)

BH4: 5' TCCCCTAGTGGGATGTGTACTTC 3' (5200 to 5222)

Or alternatively, Protocol 2

Outer primers:

MW1 : 5' CCA CAR GGA TGG AAA GGA TCA CC 3' (2997-3019)

MW2 : 5' CTG GGG CTT GTT CCA TCT RTC YTC T 3' (5575-5551)

Inner primers

INT_E : 5' ATG GGT ACC AGC ACA YAA AG 3' (4151-4160)

MW4 : 5' TAA CAC TAG GCA RAG GTG GCT T 3' (5539-5418)

Sequencing primers:

INT_E : 5' ATG GGT ACC AGC ACA YAA AG 3' (4151-4160)

SEQ INT_1: 5' GCA TAC TTC CTC TTA AAA TTA GC 3' (4521-4543)

SEQ INT_2: 5' TCC CTG TAA TAA ACC CGA AAA T 3' (4915-4897)

INT_D: 5' CAT ATG RTG TTT TAC TAA ACT 3' (5127-5107)

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

• **Conditions for RT, prot and gp120 :**

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

30' at 50°C

2' at 94°C

then: (30'' at 94°C; 30'' at 55°C; 1' 30'' at 68°C) x 40 cycles

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

12' at 94°C

then (30'' at 94°C; 30'' at 55°C; 2' at 72°C) x 40 cycles

7' at 72°C

• **Conditions for gp41 (HR1-HR2 or HR1 only):**

- **RT-PCR , with RT-PCR kit Titan (Roche):**

30' at 50°C

2' at 94°C

then : (2' at 94°C; 30'' at 55°C; 3' at 68°C) x 40 cycles

7' at 68°C

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

3' at 94°C

then (1' at 94°C; 1' at 55°C; 1' at 72°C) x 40 cycles

7' at 72°C

• **Conditions for integrase (protocol 1):**

- **RT-PCR , with RT-PCR kit Titan (Roche):**

30' at 50°C

2' at 94°C

then : (30'' at 94°C; 30'' at 55°C; 1' at 68°C) x 40 cycles

7' at 68°C

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

3' at 94°C

then (30'' at 94°C; 30'' at 55°C; 1' at 72°C) x 40 cycles

7' at 72°C

• **Conditions for integrase (protocol 2):**

- **RT-PCR , with one step super script II (invitrogen):**

30' at 45°C

2' at 95°C

then (30'' at 95°C, 45'' at 52°C, 3' at 72°C) x 40 cycles

5' at 72°C

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

2' at 95°C

then (30'' at 95°C, 45' at 52°C, 3' at 72°C) x 40cycles

5' at 72°C

Primers references:

- **RT:**

- **MJ3/MJ4 :**

Jung M, Agut H, Candotti D, Ingrand D, Katlama C, Huraux JM: Susceptibility of HIV-1 isolates to zidovudine: correlation between widely applicable culture test and PCR analysis. *J Acquir Immune Defic Syndr Hum Retrovirol* 1992, 5: 359-364

- **A35/NE1 35 and A20/NE1 20 :**

Larder BA, Kellam P, Kemp SD: Zidovudine resistance predicted by direct detection of mutations in DNA from HIV-infected lymphocytes. *AIDS* 1991, 5: 137-144.

- **Protease:**

Nijhuis M, Boucher CA, Schipper P et al: Stochastic processes strongly influence HIV-1 evolution during suboptimal protease-inhibitor therapy. *Proc Natl Acad Sci USA* 1998, 95: 1441-1446.

- **Gp41 :**

Marcelin AG, Reynes J, Yerly S, et al. Characterization of genotypic determinants in HR-1 and HR-2 gp41 domains in individuals with persistent HIV viraemia under T-20. *AIDS* 2004, 18:1340-42.

- **Gp 120:**

Delwart E. L. et al. 1993, Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. *Science* 262, 1257-61

- **Integrase:**

Malet I, Delelis O, Valantin MA et al. Biochemical characterizations of the effect of mutations selected in HIV-1 integrase gene associated with failure to raltegravir (MK-0518). XVI International HIV Drug Resistance Workshop. June 12-16, 2007. Barbados. Abstract 7.