

ANRS AC11 Resistance Study Group

PCR and Sequencing Procedures : HIV-1 Version March 2005

PCR and sequencing Primers

Reverse Transcriptase (codons 20-240):

Outer primers

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

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

Inner primers (amplification: 798 bp fragment)

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

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

Sequencing primers

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

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

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

Other set of primers which can be used for RT (aa 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'-TAATTTTTTAGGGAAGATCTGGCCTTCC-3' (bases 2082 to 2109)

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

Inner (amplification: 507 bp fragment) and sequencing primers

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

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

Other sets of primers which can be used for prot:

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'

env: gp 120 (C2V3)

Outer primers:

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

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

Inner and sequencing primers:

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

ED33: 5'-TTACAGTAGAAAATTCCCCTC-3' (bases 7360 to 7381)

env : gp41 (HR1- HR2, codons 6-172)

Outer primers :

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

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

Inner and sequencing primers :

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

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

env : 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'

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

7' à 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