

Detection of multiply spliced HIV-1 *tat* mRNA by RT-PCR *in situ* hybridization

Bruce K. Patterson

Laboratory of Viral Pathogenesis, Children's Memorial Hospital, Northwestern University Medical School, 2300 Children's Plaza #51, Chicago, IL 60614, USA.

Equipment and reagents

- ◆ Permeafix (Ortho Diagnostics, Inc.)
- ◆ RNA Core PCR Kit (Perkin-Elmer)
- ◆ Reverse transcription reaction mix (1 × RT buffer, 2.5 mM MgCl₂, 200 μM each dGTP, dATP, dCTP, 125 μM dTTP, 4 μM digoxigenin-11-dUTP, 10.0 U thermostable rTth polymerase, 40 units RNasin, 500 μM downstream primer)
- ◆ Amplification mix (1 × chelating buffer;^a 200 μM each dGTP, dATP, and dCTP; 125 μM dTTP; 4 μM dUTP-11-digoxigenin; 500 μM upstream primer; 5 units *Taq* polymerase^b)
- ◆ Digoxigenin-11-dUTP (Boehringer Mannheim)
- ◆ Primers (upstream, 5'-GCGAATTCATGGAG/TCCAGTAGATCCTAGACTA-3'; downstream, 5'-GCTCTAGACTATCTGTCCCCTCAGCTACTGCTATGG-3')
- ◆ *tat* splice junction probe (5'-TTCTCTATCAAAGCAACCCACCTCCCAATC-3') labelled at the 5'- and 3'-ends with FAM (Perkin-Elmer)
- ◆ 2 × SSC/50% formamide/500 μg/ml BSA
- ◆ 1 × SSC/50% formamide/500 μg/ml BSA
- ◆ 1 × SSC/500 μg/ml BSA

Method

- 1 Following Permeafix treatment and washes (see [Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization](#)), resuspend the cells in 40 μl of reverse transcription reaction mixture.
- 2 Incubate samples for 10 min at 70 °C and then place on ice.
- 3 Add 160 μl of PCR reaction mixture directly to the reverse transcription mixture.
- 4 Amplify the samples as described above (see [Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization](#)).
- 5 After *in vitro* amplification, centrifuge the cells as above and resuspend in 25 μl of 1 × PCR buffer.
- 6 Add 100 ng of the appropriately labelled target specific oligonucleotide probe in 10 μg/ml of sonicated herring sperm DNA to the reaction tube.
- 7 Denature the product DNA at 95 °C for 3 min then allow the target DNA to hybridize with the respective oligonucleotide probe at 56 °C for 2 h.

- 8 After hybridization, wash the cells with 1 ml of 2 × SSC/50% formamide/500 µg/ml BSA at 42 °C, with 1 ml of 1 × SSC/50% formamide/500 µg/ml BSA for 30 min at 42 °C, and 1 ml of 1 × SSC/500 µg/ml BSA^a for 30 min at ambient temperature.

Notes

- a Contains EGTA which selectively chelates manganese. This two-step reaction allows the control of the magnesium concentration in the amplification step which is a critical parameter for successful PCR *in situ* hybridization.
- b May not be necessary with all applications. PCR *in situ* hybridization, in general, requires twice as much polymerase as solution-phase PCR.