

## Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization

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### Equipment and reagents

- ◆ Permeafix (Ortho Diagnostics, Inc.)
- ◆ DNA Core PCR Kit (Perkin-Elmer)
- ◆ 1 × PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl)
- ◆ PCR reaction mixture (1 × PCR buffer; 2.5 mM MgCl<sub>2</sub>; 0.25 mM each dATP, dCTP, dGTP; 0.14 mM dTTP; 4.3 μM digoxigenin-11-dUTP; 500 μM each forward and reverse primer; 1.0 μl (10 U) *Taq* DNA polymerase)
- ◆ Digoxigenin-11-dUTP (Boehringer Mannheim)
- ◆ Target specific primers for HIV-1 (437 bp) G51 (5'-CAAATGGTACATCAGGCCATATCACCT-3') and SK39 (5'-TTTGGTCCTTGCTTATGTCCAGAATGC-3')
- ◆ HIV-1 SK19 probe (5'-ATCCTGGGATTAATAAAATAGTAAGAATGTATAGCCCTAC-3') 5'- and 3'-end-labelled with FAM (Perkin-Elmer). The 5'-end is labelled during synthesis with a FAM phosphoramidite and the 3'-end is labelled with an aminolink and FAM NHS ester (Research Genetics)
- ◆ Formamide (Life Technologies, Inc.)
- ◆ Salmon sperm DNA, sonicated (Life Technologies, Inc.)
- ◆ 2 × SSC/50% formamide/500 μg/ml bovine serum albumin (BSA)
- ◆ 1 × SSC/50% formamide/500 μg/ml BSA
- ◆ 1 × SSC/500 μg/ml BSA

### Method

- 1 Centrifuge the cells at 300–600*g* for 2 min and wash the cell pellet twice in PBS.
- 2 Fix and permeabilize the cells by resuspending with light vortexing in 50 μl of Permeafix and incubate at ambient temperature for 60 min.
- 3 Centrifuge the cells as above, wash twice with 1 ml PBS, and resuspend the cells in 190 μl of PCR reaction mixture.
- 4 Amplify the DNA in 500 μl tubes inserted into the wells of a 48-well thermocycler programmed for 25 cycles of thermal denaturation (94 °C, 1 min), primer annealing (58 °C, 2 min), and primer extension (74 °C, 1.5 min), with 5 sec added for each of 25 cycles. Run appropriate positive and negative target controls amplified with or without the addition of *Taq* DNA polymerase simultaneously with each sample.

- 5 After *in vitro* amplification, centrifuge the cells as above and resuspend in 25  $\mu$ l of 1  $\times$  PCR buffer.
- 6 Add 100 ng of the appropriately labelled target specific oligonucleotide probe in 10  $\mu$ g/ml sonicated herring sperm DNA to the reaction tube.
- 7 Denature the product DNA at 95  $^{\circ}$ C for 3 min then allow the target DNA to hybridize with the respective oligonucleotide probe at 56  $^{\circ}$ C for 2 h.
- 8 After hybridization, wash the cells with 1 ml of 2  $\times$  SSC/50% formamide/500  $\mu$ g/ml BSA at 42  $^{\circ}$ C, with 1 ml of 1  $\times$  SSC/50% formamide/500  $\mu$ g/ml BSA 30 min at 42  $^{\circ}$ C, and 1 ml of 1  $\times$  SSC/500  $\mu$ g/ml BSA<sup>a</sup> for 30 min at ambient temperature.

## Notes

- a Staining of biotinylated antibodies with reporters conjugated to streptavidin can be performed during this step for multiparameter analysis.