Detection of single-copy HIV-1 DNA using Real-Time (Taqman) PCR *in situ*

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Equipment and reagents

- STF (see Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization)
- Permeafix (see Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization)
- Xylene (see Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization)
- Ethanol (see Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization)
- Primers (see Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization)
- Probe (FAM-5'-CCTGGGATTAAATAAAATAGTAAGAATGTATAGCCCTACp-3'-TAMRA)
- Trypan Blue solution (1.0 μg/ml in PBS, pH 7.4)
- 5'-Nuclease assay reaction mix (1 × PCR buffer, 2.5 mM $MgCl_2$, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 400 μM dUTP, 0.5 U AmpErase uracil *N*-glycosylase, and 10 U AmpliTaq IS or *Taq* CS polymerase) (Perkin–Elmer)

Method

1. Cut the tissue samples to 0.5 cm thickness and fix for at least 18 h in molecular biology grade STF. Process the tissue through graded ethanols and embed in paraffin excluding any formalin fixation steps.
2. Cut tissue sections and attach them to silanized slide. [a]
3. Treat the cells and tissue sections with Permeafix for 1 h at room temperature and wash the sections twice in PBS.[b]
4. Dehydrate the cells and tissue sections through graded ethanols and air dry prior to thermal amplification as previously described (see Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization).
5. Add 50 μl of 5' nuclease reaction mixture per cell smear or tissue section. Assemble the slides using the Assembly Tool of the GeneAmp 1000 *in situ* PCR system.
6. Cycle the slides using the following profile: 50 °C for 2 min, 92 °C for 2 min followed by 30 cycles consisting of 92 °C for 1 min, 56 °C for 2 min, and 72 °C for 2 min, followed by a 4 °C soak. Perform no-*Taq*-polymerase and no-primer controls on each slide. Slides with tissue and cells lacking the desired target should be prepared identically and included with each run.

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7. Following amplification, wash the sections in PBS for 5 min at ambient temperature.
8. Fix the sections in 4% paraformaldehyde for 5 min at room temperature.
9. Wash the slides twice in PBS, pH 8.3 and counter-stain in Trypan Blue solution.
10. Place coverslips on the sections and analyse the tissue using a laser confocal microscope or monochromatic light microscope.

Notes

a. If tissue tends to fall off the slides, place one drop of 3% (v/v) white glue in water on the slide prior to adding the tissue sections. Without floating the section in a water bath, place the section on the glue bead and heat for a few seconds at 42 °C or until the tissue flattens. Do not deparaffinize. Blot excess glue with gauze. Allow to dry for at least 2 h. See Chapter 3 in PCR 3: PCR In Situ Hybridization.

b. Proteinase K can be substituted when using tissue fixed in cross-linking fixatives.

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