

PCR *in situ* hybridization for a representative tissue control (HLA-DQ α amplification)

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Equipment and reagents

- ◆ GeneAmp *In Situ* PCR System 1000 containing a thermal cycler, Assembly Tool, Disassembly Tool, AmpliCover Disc and Clips and glass slides (silane-coated, 1.2 mm thick; Perkin-Elmer).^a
- ◆ GeneAmp *In Situ* PCR Core Kit containing AmpliTaq DNA Polymerase IS, 10 × PCR Buffer, MgCl₂, and 10 mM dNTPs (Perkin-Elmer)
- ◆ PCR reaction mix (1 × PCR buffer; 3.5 mM MgCl₂; 0.25 mM each dATP, dCTP, dGTP, and dTTP; 500 μM each forward and reverse primer).
- ◆ Normal Tissue Sausage Slides (BioGenex)
- ◆ Coplin jars (Fisher)
- ◆ Histology grade 100% ethanol (Fisher)
- ◆ Xylene (BDH P/N C4330 or equivalent)
- ◆ Proteinase K (Boehringer Mannheim)
- ◆ Coverslips (Fisher)
- ◆ Digoxigenin-11-dUTP (see [Detection of single-copy HIV-1 DNA by PCR in situ hybridization](#))
- ◆ Streptavidin, alkaline phosphatase-conjugated, 750 U/mL (Boehringer Mannheim)
- ◆ Formamide (see [Detection of single-copy HIV-1 DNA by PCR in situ hybridization](#))
- ◆ Salmon sperm DNA, sonicated (see [Detection of single-copy HIV-1 DNA by PCR in situ hybridization](#))
- ◆ Humid chamber (Sigma)
- ◆ Hot temperature block (Fisher)
- ◆ Phosphate-buffered saline (PBS): 130 mM NaCl, 10 mM sodium phosphate pH 7.4; store at room temperature
- ◆ Lysis buffer; 20 mM Tris-HCl (Sigma) pH 7.4, 0.5% SDS (Gibco); store at room temperature
- ◆ Proteinase K solution (Boehringer Mannheim): 10 mg/mL in lysis buffer
- ◆ Hybridization solution: 50% formamide, 2 × SSC, 200 μg/mL sheared salmon sperm DNA, 50–250 μg biotin-labelled oligonucleotide probe (Research Genetics)
- ◆ Buffer 3: 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM MgCl₂
- ◆ 4-Nitroblue tetrazolium chloride (NBT) solution (Boehringer Mannheim)
- ◆ 5-Bromo-4-chloro-3-indoyl-phosphate (BCIP) solution (Boehringer Mannheim)

- ◆ NBT/BCIP substrate solution: add 45 μ l NBT and 35 μ l BCIP to 10 mL buffer 3; prepare fresh and store at room temperature until use
- ◆ Slide mounting medium (Fisher)
- ◆ Fast Green stain (Sigma)

Method

- 1 Deparaffinize the tissue sections through xylenes for 10 min, 100% ethanol for 10 min, 95% ethanol for 10 min.
- 2 Air dry^b and rehydrate in PBS for 5 min.
- 3 Digest with 20 μ g/ml proteinase K in 1 \times lysis buffer (20 mM Tris-HCl pH 7.4, 0.5% SDS) (must be titred) for 1 h at 37 °C. Use 500 μ l of the digestion mixture per slide. Cover the mixture gently with a coverslip that covers the length of the tissue sections.
- 4 Inactivate the proteinase K for 1 min at 95 °C by placing the slide with the coverslip on the heat block.
- 5 Wash in PBS for 1 min by placing the slide with coverslip into the buffer.^c
- 6 Dehydrate through graded ethanols (80%, 95%, and 100%) for 5 min in each dilution, then air dry as previously described.
- 7 Apply prewarmed (70 °C) reaction mix (50 μ l) to the tissue section placed on the Assembly Tool.
- 8 Add 10 units of AmpliTaq IS to the buffer bead^d on the positive control section. Create the sealed chamber using the Assembly Tool. Place the slide vertically in the GeneAmp 1000.
- 9 Amplify for 30 cycles (94 °C for 1 min, 56 °C for 2 min, 72 °C for 2 min with a 15 °C soak).
- 10 Disassemble the slides with the Disassembly Tool.
- 11 Wash once in PBS for 1 min.
- 12 Dehydrate through graded ethanols (50%, 80%, 95%, and 100%) for 5 min each.
- 13 Apply 50 μ l of hybridization mix (5 \times SSC, 50% formamide, 0.5% Tween 20, 100 μ g/ml sonicated salmon sperm DNA) with 75 ng/section^e digoxigenin- or biotin-tailed probe. Cover the slides with a coverslip.
- 14 Denature the slides for 2 min at 92 °C.
- 15 Incubate the GeneAmp 1000 overnight at 37 °C.
- 16 Wash the slides in 2 \times SSC, 0.5% Tween 20 for 10 min, 0.2 \times SSC, 0.5% Tween 20 for 10 min at room temperature.

- 17 Add 500 μ l of a 1/100 dilution of anti-digoxigenin antibody to the slides, cover each slide with a coverslip, and incubate at 4 °C for 4 h.
- 18 Wash twice in PBS for 5 min.
- 19 Incubate in buffer 3 for 2 min.
- 20 Detect with NBT/BCIP diluted in buffer 3 in the dark. Monitor the development of the purple precipitate using light microscopy.
- 21 Counter-stain with Fast Green for 30 sec or Nuclear Fast Red for 3–5 min and mount in Crystal Mount or other aqueous mountant.

Notes

- a See Chapter 9 in [PCR 3: PCR *In Situ* Hybridization](#).
- b Tissue appears pure white rather than grey when the tissue is appropriately air dried.
- c Allow the coverslip to fall off the slide passively. If the coverslip does not fall off within a few seconds, gently dip the slide up and down in the PBS.
- d If the buffer bead is off-centre, place the polymerase aliquot over the centre of the tissue section and the buffer bead will migrate back towards centre. This technique will minimize bubble formation.
- e The probe must be titred; use the concentration of probe that does not give signal when hybridized to an uncycled tissue of interest.