

Specific detection of double-stranded DNA sequences in Lowicryl K4M sections by *in situ* hybridization

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Equipment and reagents

- ◆ Incubator at 37°C
- ◆ Gold grids bearing Lowicryl K4M sections
- ◆ Protease (or proteinase K)
- ◆ Nuclease S1 from *Aspergillus oryzae* (commercial stock solution at 400 U/ml)
- ◆ 10 x nuclease S1 buffer: 0.33 M sodium acetate, 0.5 M sodium chloride, 0.3 mM zinc sulfate pH 7.5
- ◆ 0.5 M NaOH (from a 5M NaOH stock)
- ◆ Hybridization solution
- ◆ Gold labelled anti-biotin (or antidigoxigenin) antibody
- ◆ 5% aqueous uranyl acetate solution

Method^a

- 1 Collect ultrathin Lowicryl K4M sections of fixed formaldehyde-material^b (see [Successive steps for Lowicryl K4M embedding and ultrathin sectioning of somatic mammalian cells prior to *in situ* hybridization](#)) on Formvar carbon coated gold grids (see [Preparation of Formvar carbon coated grids for ultrathin sections of Lowicryl embedded cells prior to *in situ* hybridization](#)).
- 2 Digest proteins by a protease (or proteinase K) treatment of the grids (see [Enzymatic digestions of Lowicryl ultrathin sections prior to post-embedding hybridization](#)).
- 3 Digest single-stranded nucleic acid molecules of the ultrathin sections with nuclease S1 (1600 U/ml for 1 h at 37°C) (see [Enzymatic digestions of Lowicryl ultrathin sections prior to post-embedding hybridization](#)).^c
- 4 Denature double-stranded DNA of the sections by a NaOH treatment of grids (0.5 M NaOH for 4 min at room temperature) (see [Denaturation of DNA in ultrathin sections of Lowicryl K4M embedded material](#)).
- 5 In parallel denature the double-stranded DNA of the hybridization solution by a 4 min heat treatment (see [Hybridization solution: composition, heat treatment prior to post-embedding *in situ* hybridization](#)).

- 6 According to [Post-embedding *in situ* hybridization and detection of hybrids](#), form hybrids at the surface of the sections (incubation of grids in the presence of the heat-treated hybridization solution for 60 to 90 min at 37°C), detect the hybrids by immunogold labelling, and then stain the grids prior to EM observation.

Notes

- a Exclusive detection of duplex DNA sequences in cells containing single-stranded and double-stranded DNA molecules requires prior elimination by S1 nuclease of all single-stranded nucleic acid molecules, both DNA and RNA. This protocol has given satisfactory data to determine the respective amounts of double-stranded and single-stranded viral DNA molecules in the different substructures of the nuclei of cells infected with herpes simplex virus and adenovirus.
- b We found that formaldehyde fixation of cells, but not glutaraldehyde fixation, allows the detection of double-stranded DNA molecules, either cellular or viral DNA, on sections previously digested with protease and S1 nuclease and treated with NaOH.
- c Total removal of single-stranded nucleic acid sequences requires prior elimination of the proteins because specific DNA and RNA binding proteins protect them.