

Post-embedding *in situ* hybridization and detection of hybrids

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Equipment and reagents

- ◆ Parafilm
- ◆ Incubator at 37°C, 55°C or 64°C
- ◆ Gold grids bearing Lowicryl K4M sections
- ◆ Probe-containing hybridization solution
- ◆ Phosphate buffered saline (PBS)
- ◆ Gold labelled anti-biotin (or anti-digoxigenin) antibody
- ◆ 5% aqueous uranyl acetate solution

Method^a

- 1 Collect ultrathin Lowicryl K4M sections of fixed material (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 Denature the probe just before use by heating the hybridization solution (see [Hybridization solution: composition, heat treatment prior to post embedding *in situ* hybridization](#)).
- 3 Distribute 1–2 µl drops of freshly denatured hybridization solution on a sheet of Parafilm placed in a wet chamber.
- 4 Float the grids bearing Lowicryl K4M sections, with or without pre-treatments (see [Enzymatic digestions of Lowicryl sections prior to post-embedding hybridization](#) and [Denaturation of DNA in ultrathin sections of Lowicryl K4M embedded material](#)), on the surface of the probe-containing drops.
- 5 Place the wet chamber in an incubator for 1–4 h at a previously defined temperature.^b
- 6 Rinse the grids at room temperature for about 1 min by rapid passages over three drops of cold PBS distributed on a sheet of Parafilm
- 7 Float the grid-mounted sections on 5 µl drops of anti-biotin (or anti-digoxigenin) antibody conjugated to gold particles, 10 nm in diameter, diluted 1:25 in PBS, at room temperature for 30 min.

- 8 Rinse the grids as above by floating them briefly on three droplets of cold PBS.
- 9 Wash the grids in a jet of distilled water.
- 10 Air dry the grids.
- 11 Stain the grids for 10 min with 5% aqueous uranyl acetate solution before transmission EM observation.^c
- 12 Store the grids up to one year in darkness for further observations.

Notes

- a The post-embedding *in situ* hybridization protocols that we have employed for detecting different types and forms of nucleic acid sequences contain some identical steps but vary in other crucial details depending on the nucleic acid sequence and biological system under study. The protocols [Successive steps for specific detection of DNA sequences by post-embedding *in situ* hybridization](#) and [Specific detection of double-stranded DNA sequences in Lowicryl K4M sections by *in situ* hybridization](#) give the conditions under which we observed best results for detection of DNA (concomitantly double-stranded and single-stranded DNA) whereas the protocols [Successive steps for specific detection of DNA sequences by post-embedding *in situ* hybridization](#) without step 3 and [Successive steps for specific detection of RNA molecules in Lowicryl K4M sections by *in situ* hybridization](#) are related to the exclusive detection of single-stranded DNA and RNA, respectively.
- b The hybridization step is similar whatever the pre-treatment of sections although some conditions vary, including the temperature and the duration of the hybridization. As a guide, 37°C for 60 to 90 min allows the detection of *Alu* sequences, ribosomal genes and adenovirus and herpes simplex virus genomes whereas 37°C for 90 min to 3.5 h allows the detection of poly(A) tails, U3 small nucleolar RNA and viral RNA. Higher temperatures (55°C and 64°C) are required for the detection of small nuclear (U1 and U2) RNA and ribosomal RNA.
- c Post-embedding *in situ* hybridization preserves the fine structure well and permits the clear identification of the structures containing the target under study. Its limitation is that only targets which are exposed at the surface of the ultrathin section bind the probe.