

## Successive steps for specific detection of RNA molecules in Lowicryl K4M sections by *in situ* hybridization

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### Equipment and reagents

- ◆ Incubator at 37°C, 55°C and 64°C
- ◆ Gold grids bearing Lowicryl K4M sections
- ◆ Protease (or proteinase K)
- ◆ DNase I
- ◆ Probe-containing hybridization solution
- ◆ Gold labelled anti-biotin (or anti-digoxigenin) antibody
- ◆ 5% aqueous uranyl acetate solution

### Method

- 1 Collect ultrathin Lowicryl K4M sections of either formaldehyde- or glutaraldehyde-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 Depending on the model system under study, if required digest ultrathin sections with protease (0.2 mg/ml for 15 min)<sup>a</sup> and/or DNase I (1 mg/ml for 1 h) at 37°C (see [Enzymatic digestions of Lowicryl sections prior to post-embedding hybridization](#)).<sup>b</sup>
- 3 Heat treat the hybridization solution if it contains double-stranded DNA molecules (see [Hybridization solution: composition, heat treatment prior to post-embedding \*in situ\* hybridization](#)).
- 4 Incubate the grids in the presence of the heat-treated hybridization solution at a selected temperature and duration depending on the GC content of the probe (see [Hybridization solution: composition, heat treatment prior to post-embedding \*in situ\* hybridization](#)).<sup>c</sup> As a guide, 37°C for 90 min to 3.5 h allows the detection of poly(A) tails, U3 small nucleolar RNA, adenovirus and herpes simplex virus RNA, 55°C is required for detecting U1 and U2 small nuclear RNA, and 64°C allows the detection of ribosomal RNA.

- 5 Reveal the hybrids and stain the grids before observation (see [Post-embedding \*in situ\* hybridization and detection of hybrids](#)).

## Notes

- a Elimination of the proteins of the section prior to the hybridization step improves the accessibility of a defined RNA target sequence to the probe. In addition, it suppresses false positive results due to the binding of the probe to some proteins.
- b Some model systems contain single-stranded DNA segments, such as cells infected with herpes simplex virus type 1 and adenovirus type 5, which are revealed concomitantly with the related RNA sequences. Specific detection of the viral RNA in such biological materials requires the previous elimination of DNA by a DNase treatment.
- c Temperature and duration of the hybridization are adjusted empirically to maximize the hybridization signal. We have chosen to keep constant the ionic strength and the formamide concentration of the hybridization solution and to adjust stringency by varying the hybridization temperature.