

Successive steps for Lowicryl K4M embedding and ultrathin sectioning of somatic mammalian cells prior to *in situ* hybridization

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Equipment and reagents

- ◆ 0.1 M Sørensen phosphate buffer, pH 7.2-7.3: 13.67 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 3.62 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$. Add H_2O to 1 litre
- ◆ Fixatives: 4% formaldehyde (depolymerized paraformaldehyde) or 1.6% glutaraldehyde (from a 25% stock solution) in Sørensen phosphate buffer (make up fresh each time)
- ◆ Methanol: stocks of 30% and 50% stored at 4°C, stocks of 70% and 90% stored at -20°C
- ◆ Lowicryl K4M embedding solution (from a Lowicryl K4M kit) – stored at -20°C for a few days: 4 g cross-linker, 26 g monomer, and 0.15 g initiator
- ◆ Gelatin capsules
- ◆ Deep-freeze
- ◆ Fluorescence tubes (6 W, 360 nm UV light)
- ◆ Ultramicrotome fitted with a diamond knife
- ◆ 200 mesh gold grids

Method

The protocol is appropriate to studies of small tissue samples.

- 1 Fix monolayer of somatic mammalian cells in formaldehyde or glutaraldehyde for 1 h at 6-8°C.^a
- 2 Detach the cells from the culture dishes, centrifuge at 5,000 g for 15 min and rinsed the resulting pellets in Sørensen phosphate buffer for 2 h or more.
- 3 Dehydrate the pellets successively with 30% and 50% methanol, each for 5 min at 4 °C, then with 70% methanol for 5 min and 90% methanol for 30 min, each at -20°C.^c
- 4 Impregnate the pellets with Lowicryl embedding solution through 1:1 (v/v) 90% methanol: Lowicryl K4M for 1 h, followed by 1:2 (v/v) 90% methanol: Lowicryl K4M for 1 h, each at -20°C.
- 5 Plunge samples in Lowicryl K4M embedding solution, first for 1 h, then in a second Lowicryl-bath overnight, each at -20 °C.

- 6 Embed in Lowicryl K4M embedding solution using gelatin capsules, and store overnight at -20°C .
- 7 Polymerize under long wavelength UV light for five days in a deep-freeze at -30°C and then for 24 h at room temperature by the use of four fluorescence tubes arranged in a sandwich fashion at 30 cm distance from the capsules.
- 8 Cut ultrathin sections with an ultramicrotome fitted with a diamond knife and collect the sections on gold grids coated with a Formvar film strengthened by carbon. See [Preparation of Formvar carbon coated grids for ultrathin sections of Lowicryl embedded cells prior to *in situ* hybridization](#).
- 9 Store grids bearing Lowicryl sections in darkness until use for *in situ* hybridization (up to two years). See [Post-embedding *in situ* hybridization and detection of hybrids](#).

Notes

- a Formaldehyde fixation is required for the detection of double-stranded DNA by *in situ* hybridization whereas formaldehyde and glutaraldehyde fixations allow the detection of RNA sequences.
- b Methanol is preferred to ethanol and acetone because no artefactual aggregation of DNA, especially of viral DNA, occurs with methanol.