Preparation of Formvar carbon coated grids for ultrathin sections of Lowicryl embedded cells prior to *in situ* hybridization

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Equipments and reagents

- Glass slides and glass container
- Gold grids, 200 mesh
- Carbon evaporator
- 1,2-dichloroethane
- Formvar: stock solution at 0.25% in water-free 1,2-dichloroethane, stored at room temperature and in darkness for several months

Method[a]

1. Filter the Formvar solution just before use.
2. To prepare the film, dip a glass slide into the solution, remove it slowly, and dry in a vertical position.
3. Float the film off the slide on to a water surface after scraping the edges of the slide, arrange the 200 mesh gold grids[b] on the floating film, recover the film plus grids onto a piece of filter paper, and finally, pick up the paper carrying the film and the grids with forceps.
4. After air drying, evaporate a thin layer of carbon on the Formvar coated grids in order to stabilize the plastic film. The coated grids can be stored up to several months in a Petri dish prior to be used for enzymatic digestions (see Enzymatic digestions of Lowicryl sections prior to post-embedding hybridization), NaOH treatment (see Denaturation of DNA in ultrathin sections of Lowicryl K4M embedded material) and *in situ* hybridization (see Post-embedding *in situ* hybridization and detection of hybrids).

Notes

a Grids coated with a Formvar film strengthened by carbon are used for post-embedding *in situ* hybridization because of the high stability of the ultrathin sections under the beam even after additional extraction treatments, and the good conservation of the samples which can be re-examined even after one or two years. Ultrathin sections also can be recovered on naked grids but such samples

are very weak and often become altered and torn during the successive steps of the post-embedding *in situ* hybridization.

b Grids of gold or nickel are required for post-embedding *in situ* hybridization. Copper reacts with the components of the hybridization solution therefore copper grids never can be used.