Successive steps for specific detection of DNA sequences by post-embedding *in situ* hybridization

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Equipment and reagents

- Incubator at 37°C
- Gold grids bearing Lowicryl K4M sections of formaldehyde-fixed material
- Protease (or proteinase K)
- RNase A
- 0.5 N NaOH (from a 5N NaOH stock)
- Probe-containing hybridization solution
- Gold labelled anti-biotin (or anti-digoxigenin) antibody
- 5% aqueous uranyl acetate solution

Method[a]

1. Collect ultrathin Lowicryl K4M secetions of formaldehyde-fixed 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. Eliminate successively the proteins and the RNA of the sections (see Enzymatic digestions of Lowicryl ultrathin sections prior to post-embedding hybridization).[c]

3. Denature the double-stranded DNA of the ultrathin sections with 0.5 N NaOH for 4 min at room temperature (see Denaturation of DNA in ultrathin sections of Lowicryl K4M embedded cells).[d]

4. In parallel, denature the double-stranded DNA of the hybridization solution (see Hybridization solution: composition, heat treatment prior to post-embedding *in situ* hybridization).

5. Float the protease-RNase-NaOH pre-treated grids on the surface of drops of heat treated hybridization solution for 60 to 90 min at 37°C then detect the hybrids formed at the surface of the ultrathin sections by immunogold labelling (see Post-embedding in situ hybridization and detection of hybrids).

6. Stain the grids for 10 min with 5% aqueous uranyl acetate staining prior to EM observation.

Notes

a This protocol has contributed to localize the ribosomal genes and the telomeric DNA as well as to characterize the respective distribution of cellular *Alu* elements and viral genomes in cells infected with either adenovirus type 5 or herpes simplex virus type 1 in somatic mammalian cells. When step 3 is omitted, the protocol allows the exclusive detection of single-stranded DNA molecules.

b Under our experimental conditions, formaldehyde fixation is required to obtain extensive NaOH denaturation of double-stranded DNA.

c Protease and RNase digestion of sections facilitates the access of the DNA target to the probe and prevents concomitant detection of related RNA sequences, respectively.

d In certain models such as in cells infected with adenovirus and herpes simplex virus, portions of viral single-stranded DNA are normally present in the cells which can be specifically detected by omitting the step 3. As a result, only molecules which are normally single-stranded can bind the probe. Since RNA molecules are digested by the RNase A pre-treatment of sections, only single-stranded DNA molecules can react with the probe. This modified protocol has contributed to the precise localization of viral single-stranded DNA fragments among the innumerable double-stranded viral genomes present in the infected nuclei.