

Laboratory for Advanced Electron and Light Optical Methods

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DNA (Plasmid) Preparation for TEM

(from Dykstra, M.J. 1993. *A Manual of Applied Techniques for Biological Electron Microscopy*. Plenum Press, NY)

Courtesy Chris Dykstra, University
of North Carolina at Chapel Hill

1. Application and Objectives: This is a quick method for the preparation of purified short segments of nucleic acids (e.g., plasmids) for visualization in a transmission electron microscope. The technique confers sufficient bulk and contrast to isolated short pieces of nucleic acids for the purpose of measuring their length or assessing whether they are single-stranded or double-stranded utilizing a transmission electron microscope.

2. Materials Needed:

1 mg/ml cytochrome c (Sigma Type VI) in distilled water
0.25 M ammonium acetate in distilled water, pH 7.5
distilled water
50% ethanol
0.5-5.0 µg/ml nucleic acid in distilled water
ParafilmTM
Petri dishes
micropipets (2-10 µm and 10-100 µm adjustable) and tips
4% uranyl acetate stock (alcoholic or aqueous)
200 mesh Formvar-coated grids
vacuum evaporator with flat rotary stage
double-stick tape
1.5 cm platinum-palladium wire
microfuge tubes
shell vial or scintillation tube for mixing uranyl acetate
forceps
Whatman #1 filter paper (9 cm disks)

3. Procedure:

- 3.1. Mix the following in a microfuge tube:
 - 40 μL 0.25 M ammonium acetate, pH 7.5
 - 10 μL 0.5-5.0 $\mu\text{g/ml}$ DNA in distilled water
- 3.2. Let the mixture sit 1 min in the microfuge tube.
- 3.3. To the microfuge tube, add 1.0 μL of 1 mg/ml cytochrome C (Sigma Type VI) in water. Invert capped tube several times.
- 3.4. Place 51 μL of solution from microfuge tube onto ParafilmTM in a Petri dish, cover the dish and wait for 1 min.
- 3.5. Touch the Formvar-coated side of a grid to the drop (more than one grid can be touched to the same drop).
- 3.6. Immediately stain in dilute uranyl acetate in 50% ethanol (prepared by adding one drop of normal post-stain stock to 20 ml ethanol in a shell vial) by dipping the grid into the shell vial of uranyl acetate in ethanol for 5 sec.
- 3.7. To destain the stained grid, dip the stained grid into 50% ethanol for 5 sec immediately after it is removed from the staining mixture.
- 3.8. Dry the destained grid quickly and completely with a piece of filter paper (see the section on post-staining for drying procedure) after destaining.
- 3.9. After 15 min, stick the edge of the grid specimen-side up to a piece of double-stick tape previously applied to the surface of the horizontally-rotating specimen support in the vacuum evaporator. Put the grid near the center of the rotating stage and situate the center of the stage 1.5 cm below and 8 cm horizontally away from the platinum-palladium source (this gives a 10° shadowing angle).
- 3.10. Pump the vacuum evaporator chamber down to $2-5 \times 10^{-6}$ Torr and then fuse the platinum-palladium wire with the tungsten basket as described in the instructions for shadowing.
- 3.11. Turn on the rotary stage and allow to rotate at 100 rpm.
- 3.12. Fire the platinum-palladium-coated tungsten basket as described under shadowing and remove the grid from the vacuum chamber.

3.13. Examine the coated nucleic acid with a TEM. The nucleic acids should be easily seen at a TEM magnification of 12,000 X or above.

4. Results Expected: The nucleic acids should be well spread out on the surface of the coated grid and should have adequate bulk to be seen easily (Fig. 60). The metal coating should not be objectionably grainy.

5. Cautionary Statements: All cytochrome C is not the same. The cytochrome C is used to spread the nucleic acid over the drop to which the coated grid is touched. If the nucleic acid is not evenly spread over the grid surface, try another production batch of cytochrome C. Once a satisfactory stock of cytochrome C is demonstrated, it is good practice to make up aliquots of appropriate quantity in microfuge tubes and to store them frozen at -20° C until needed.

Before proceeding with this method, review the instructions for shadowing and the specific instructions for your vacuum evaporator. Also review the cautionary statements about uranyl acetate provided in the section on post-staining.