

Laboratory for Advanced Electron and Light Optical Methods

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I. Preparation of Rotary-Shadowed, Low Angle Samples (DNA, etc.)

A. Purpose: Low-angle rotary shadowing can bulk up small, linear molecules such as DNA or structures such as bacterial pili. The width of the materials increases because of the evaporated metal deposited on their surfaces, but the length does not increase appreciably. The added width makes the linear structures easier to see, and the metal replica produced is extremely stable under the electron beam and can be stored for extended periods of time, if necessary.

B. Procedure:

1. A suspension of particulates is placed onto a Formvar-coated grid, allowed to stand at room temperature for about 5 min, and then wicked dry with a piece of Whatman #1 filter paper. With a low-angle shadowing of macromolecules, the biological material remains on the grid, while with the high-angle shadowing technique described below, the biological material is digested from the replica before the sample is viewed in the transmission electron microscope.
2. After the grid has totally air-dried, it is placed within the vacuum evaporator on a piece of double-stick tape on a rotating stage. The center of the stage should be approximately 1 cm below the tungsten basket containing a balled-up piece of platinum or platinum-palladium wire that is about 1.5 cm long. The center of the stage should be positioned 8 cm horizontally away from the tungsten basket containing the wire.
3. Prepare the vacuum evaporator for a metal and carbon evaporation, pump the bell jar down to somewhere in the 10^{-6} Torr range, and select the electrode set with the tungsten basket.
4. Heat up the tungsten wire gradually, while observing it through a dark piece of radiographic emulsion. When the platinum or platinum-palladium wire melts, quickly increase the amperage to about 20-22 A. **Once the noble metal melts, it must be burned off quickly because the molten metal will degrade the tungsten basket and cause it to break, ending the evaporative process prematurely.**
5. After the wire has been evaporated, evaporate the carbon source to provide for more stability and conductivity to the sample surface when it is exposed to the TEM beam.
6. After finishing the evaporative process, follow the SOPs for the evaporator being used to remove the grids, clean the system, pump it down again, and shut it off.

II. Davis Technique for DNA (R.W. Davis, D. Botstein, J.R. Roth. 1980. A Manual for Genetic Engineering. Advanced Bacterial Genetics. Cold Spring Harbor, NY. 254 p.)

A. Materials Needed:

5 M NH₄Ac
1 mg/ml cytochrome C
25 ng DNA
0.05 M uranyl acetate
0.05 M HCl
90% ethanol
isopentane

B. Procedure:

1. Mix 3.5 µL of 5 M NH₄ Ac with 1 mg/ml cytochrome C
2. Add 25 ng DNA and water to bring final volume to 50 µL
3. Put the mixture on parafilm; touch a Formvar-coated grid to the side of the drop
4. Dry the grid almost to dryness with Whatman#1 filter paper and dip in uranyl acetate stain (10 µL of uranyl acetate/HCl added to 10 ml of 90% ethanol) for about 30 sec (made up right before use)
5. Rinse for about 5 sec in isopentane
6. Rotary shadow with PT-PD at 10° as described above

III. Micromethod for spontaneous absorption (Sommerville, J., and U. Scheer. 1987. Electron Microscopy in Molecular Biology: A practical Approach. IRL Press, Oxford. 248 p.)

A. Materials Needed:

1. Stock Solutions:

0.2 µg/ml DNA
2.6 µg/ml cytochrome c in 0.3 M ammonium acetate pH 6.0
(can be stored at about 4° C)
(if ssDNA is to be visualized, 30-50% formamide may be added, but check that pH doesn't change with time)
50 mM uranyl acetate (0.212 g uranyl acetate in 95% alcohol);
filter and store at about 4° C in the dark
20% formaldehyde

2. To Make Working Solution:

To make final working solution containing 0.1 µg/ml DNA, 1.3 µg/ml cytochrome C, 0.15 M ammonium acetate pH 6.0, mix the following **in order**:

a. Mix the following as a stock:

10 ml 0.3 M ammonium acetate pH 6.0
26 µg cytochrome c (Sigma Equine Type VI, C-7752)

(store at about 4° C)

b. Add:

100 µL cytochrome c/ammonium acetate stock (1 above)
1 µL 20% formaldehyde

c. Mix:

25 µL of cytochrome c/ammonium acetate/formaldehyde
(from 2a and 2b above) with 25 µL of a 0.2 µg/ml DNA

-The final working solution is 0.1 µg DNA/1.3 µg cytochrome c in 1 ml of
0.15 M ammonium acetate buffer pH 6.0
containing 0.2% formaldehyde (70 mM)

3. Procedure:

- a. Place 40 µL drop of working mixture on clean Parafilm surface.
- b. Cover and let sit for 4 min to several hours (for long periods, set up as a moist chamber).
- c. Touch formvar-coated grid to drop.
- d. Stain grid in uranyl acetate solution for 20-30 sec.
- e. Rinse grid in 95% ethanol for 10-15 sec.
- f. Dry grid.
- g. Rotary shadow the grid at a 10° angle with 1.5 cm of platinum palladium (8 mil) wire and carbon coat as described above.

IV. DNA Plasmid Preparation for TEM (From C.C. Dykstra)

A. Procedure:

1. Mix the following:
 - 40 µL 0.25 M ammonium acetate, pH 7.5
 - 10 µL 0.5-5.0 µg/ml DNA in distilled water
2. Let sit about 1 min in tube
3. Add 1.0 µL of 1 mg/ml cytochrome C (Sigma Type VI) in water
4. Place 51 µL drop on parafilm and let sit about 1 min
5. Touch Formvar-coated grid to drop (more than one can be done)
6. Stain in dilute uranyl acetate in 50% ethanol (one drop of normal post-stain stock in 20 ml ethanol)
7. Destain in 50% ethanol
8. Dry and shadow at a 10° angle as described above on a rotating stage, with platinum or platinum-palladium
9. Examine with TEM above 12,000X or so