Microtechnique for electron microscopy of DNA

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In recent studies on kinetoplast DNA, the mitochondrial DNA of trypanosomatids (1), we had insufficient quantities for standard electron microscopy (EM) procedures. We therefore developed a new microtechnique for sample preparation. EM of DNA is commonly done by the Kleinschmidt method (2), in which the nucleic acid is first adsorbed to a positively charged protein monolayer formed at an air-water interface. The spread DNA is then picked up on a film (e.g. nitrocellulose) supported on an EM grid. Using a common modification of the Kleinschmidt method, by Davis *et al.* (3), this technique requires about 25 ng of plasmid DNA. Since kinetoplast DNA is in the form of a large network of several thousand minicircles, the requirements are closer to 50–100 ng. Our new method is a variation of the Davis

technique using much smaller amounts of DNA for each spreading. Furthermore, this technique allows preparation of grids for multiple samples in considerably less time than required by traditional methods while maintaining the quality of the micrographs. Therefore, we now use this method even if we have abundant supplies of DNA. The method should be applicable for any DNA, especially when it is available only in small quantities (e.g. from microorganisms that are difficult to culture or from *in vitro* enzyme reactions).

Spreadings were conducted in a Teflon block (12.5 cm × 8.5 cm × 1.3 cm) drilled with 50 cylindrical holes, each 6.3 mm in diameter and 9.3 mm in depth. The holes were spaced in the block in 5 rows with 10 holes per row. Commercially available

Figure 1. Electron micrograph of *Crithidia fasciculata* kinetoplast DNA prepared by the microtechnique. Bar is 1 μ m.

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microtiter plates were unreliable for this technique because DNA was frequently lost by adsorption on the plastic surface. Since grids can be prepared from two samples at a time, two wells were each filled with 320 μ l of hypophase (10% formamide (EM Science), 10 mM tris-HCl, pH 8.6), which bulged slightly above the surface. To remove any dirt, the hypophase surface was wiped gently with a Teflon bar or a small piece of Parafilm, although omission of this cleaning procedure did not seem to affect the results.

The spreading solution was prepared by placing 1 or 2 μ l of DNA solution (0.4 ng/ μ l for a plasmid or 2 ng/ μ l for kinetoplast DNA networks) in TE buffer on a piece of Parafilm. This solution was mixed with an equal volume of 80% formamide, 0.2 M Tris-HCl, pH 8.6, 0.2 mg/ml cytochrome c (horse heart, Sigma Type VI). A sample of this solution (about 0.3 μ l) was then picked up with a P10 Pipetman with a 10 μ l disposable tip and carefully expelled to form a tiny drop on the tip. This drop was gently placed on the hypophase surface and left undisturbed for one minute to allow the DNA to spread. Then DNA-cytochrome film was picked up from the hypophase surface by touching it gently with a nitrocellulose-covered grid (prepared according to ref. 4) held in locking tweezers. The grid was dipped for 10 seconds in 90% ethanol, stained with uranyl acetate (50 μ M in 90% ethanol) for 30 seconds, and rinsed with 90% ethanol for 10 seconds. After draining the ethanol excess on a Whatman #1 filter paper, the grids were dried at room temperature and rotary shadowed (8° angle) with Pt-Pd (80–20 alloy) by standard procedures (5). Typically, about 20–30 samples can be prepared by this method in about 1 hour, providing that two are spread at once; the traditional method takes over 5 minutes per sample. Two grids, from different spreading solutions, can be prepared at the same time if there is a 50 second interval between spreading the first and second sample. Also several grids can be picked up from the same sample; we wait 3 minutes between successive lifts. Because there are 50 wells in the Teflon block, it is not necessary to clean the apparatus (by extensive rinsing with distilled water) until 50 grids have been prepared. Figure 1 shows a sample of *Crithidia fasciculata* kinetoplast DNA prepared for EM by this method, and other examples are in a recent publication (6). Another micromethod based on the Kleinschmidt procedure has been described by Inman and Schnöss (7).

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