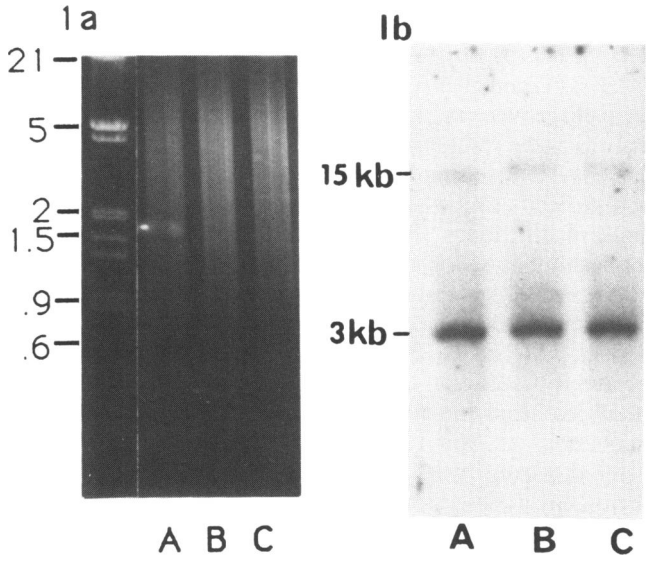
A simple method for the preparation of chromosomal DNA from cell culture

Hong-wu Xu, Anthony M.Jevnikar and Vicki E.Rubin-Kelley*
The Laboratory of Immunogenetics and Transplantation, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

Submitted July 6, 1990

There are a number of different procedures of various complexity, for the preparation of genomic DNA (1–3). We describe a very simple and rapid method of obtaining high quality genomic DNA. Our method also avoids the hazard of organic solvent extraction and does not require centrifugations, or mechanical manipulations such as spooling DNA on a pasteur pipette which may result in shearing or loss of material. Time consuming dialysis is also not required. A 100-mm plate of proximal tubular epithelial cells was washed twice with PBS. Cells were lysed with 2 ml of 6 M guanidine HCl (M_r = 95.6), 0.1 M NaOAc. The lysed cell suspension was then carefully layered under 10 ml of EtOH in a 15 ml polystyrene tube using a large bore pasteur pipette wetted with EtOH for transfer. The DNA was then recovered by rolling the tube horizontally. This not only reduced DNA physical shearing to a minimum but also increased the contact area between the two phases. As the DNA condensed, the tube was gently inverted several times until the EtOH and aqueous phases were thoroughly mixed. After extracting one more time with EtOH, the DNA was dissolved in 2 ml of 1×TE buffer, with 2% SDS, 1 mg DNase free RNase and 100 μg proteinase K and incubated at 37°C for at least 30 min (or overnight). After this, 10 ml of EtOH was added carefully and the DNA was recovered as above. Finally, the DNA was transferred to a fresh microcentrifuge tube containing 1 ml of TE (10 mM Tris, 0.1 mM EDTA, pH 7.5–8.0), using a large bore pipette wetted with TE buffer. The tube was incubated at 65°C for 30 min and then transferred to 4°C on a rocking platform until the DNA was completely dissolved. This often takes 1–3 days. Due to the high purity of the DNA it is helpful to dissolve DNA at a lower concentration (10–15 ng/ml).

Gel electrophoresis and staining with ethidium bromide (Fig. 1a) indicated that the DNA isolated by this procedure is directly restriction endonuclease digestible (HindIII/PstI) and not contaminated with RNA. It also gave an excellent result in Southern hybridization (Fig. 1b). The general size of the DNA obtained by this method is greater than 80 kb, and the ratio of OD_{260} to OD_{280} was nearly 2.



REFERENCES

1. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning*, 2nd edition. Cold Spring Harbor Laboratory Press, NY.
2. Ausubel,F.M., Brent,R., Kingston,R.E., Moore,D.D., Seidman,J.G., Smith,J.A. and Struhl,K. (1987) *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience.
3. Bowtell,D.D.L. (1987) *Anal. Biochem.* **162**, 463.

* To whom correspondence should be addressed