A simple salting out procedure for extracting DNA from human nucleated cells

S.A.Miller, D.D.Dykes and H.F.Polesky

Memorial Blood Center of Minneapolis, 2304 Park Avenue South, Minneapolis, MN 55404, USA
Submitted November 18, 1987

One of the obstacles encountered when extracting DNA from a large number of samples is the cumbersome method of deproteinizing cell digests with the hazardous organic solvents phenol and isochloroform. Several other non-toxic extraction procedures have been published, but require either extensive dialysis (1) or the use of filters (2). A rapid, safe and inexpensive method was developed to simplify the deproteinization procedure. This method involves salting out of the cellular proteins by dehydration and precipitation with a saturated NaCl solution.

Buffy coats of nucleated cells obtained from anticoagulated blood (ACD or EDTA) were resuspended in 15 ml polypropylene centrifugation tubes with 3 ml of nuclei lysis buffer (10 mM Tris-HCl, 400 mM NaCl and 2 mM Na_2EDTA, pH 8.2). The cell lysates were digested overnight at 37°C with 0.2 ml of 10% SDS and 0.5 ml of a protease K solution (1 mg protease K in 1% SDS and 2 mM Na_2EDTA). After digestion was complete, 1 ml of saturated NaCl (approximately 6M) was added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 2500 rpm for 15 minutes. The precipitated protein pellet was left at the bottom of the tube and the supernatant containing the DNA was transferred to another 15 ml polypropylene tube. Exactly 2 volumes of room temperature absolute ethanol was added and the tubes inverted several times until the DNA precipitated. The precipitated DNA strands were removed with a plastic spatula or pipette and transferred to a 1.5 ml microcentrifuge tube containing 100-200 µl TE buffer (10 mM Tris-HCl, 0.2 mM Na_2EDTA, pH 7.5). The DNA was allowed to dissolve 2 hours at 37°C before quantitating.

The DNA obtained from this simple technique yielded quantities comparable to those obtained from phenol-chloroform extractions. The 260/280 ratios were consistently 1.8-2.0, demonstrating good deproteinization. Restrictions were performed using a number of different enzymes requiring high, medium or low salt concentrations, all resulting in complete restriction. This procedure has been used in our laboratory on several thousand blood samples for parentage, population and forensic studies. This technique is used with our non-isotopic hybridization procedures (3) rendering the entire process of RFLP analysis free of toxic materials.

REFERENCES:

1. Longmire, J., Albright, K., Lewis, A., Meincke, L. and Hildebrand, C. (1987) Nucleic Acids Res. 15, 859.
2. Leadon, S. and Cerutti, P.(1982) Anal. Biochem. 120, 282-288.
3. Dykes, D., Fondell, J., Watkins, P. and Polesky, H. (1986) Electrophoresis 7, 278-282.