A simple and efficient non-organic procedure for the isolation of genomic DNA from blood

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The isolation of genomic DNA from blood typically involves digestion of nuclei with a combination of Proteinase K and SDS followed by deproteinization with organic reagents such as phenol and chloroform. Additional purification steps such as extensive dialysis, precipitation with a saturated solution of NaCl and/or absolute ethanol are then required for enzymatic analysis of the extracted DNA (1). The isolation procedure described here is both simple and rapid, eliminating the necessity for hazardous organic reagents. The method involves the incubation of nuclei with only Proteinase K at 65°C. It has been shown that Proteinase K is more active on denatured protein and that after prolonged incubation at 65°C it autoinactivates (2). As a result, following a 2 hour incubation, the extracted DNA can be used directly for enzymatic analysis without any additional purification.

Nuclei were prepared by mixing an aliquot of whole blood with a 4 fold excess of ice cold CLB (0.32 M Sucrose, 10 mM Tris-HCl pH 7.6, 5 mM $MgCl_2$, 1% Triton X-100). The lysate is centrifuged at 900 X g for 5 minutes at 4°C. The pellet is washed with CLB and following centrifugation the nuclear pellet is rinsed with a small aliquot of cold PLB (10 mM Tris-HCl pH 8.0, 10 mM NaCl, 10 mM EDTA). For 1 ml of blood, the nuclei are resuspended in 250 ul of PLB+ ProK (PLB with 1 mg/ml Proteinase K) and incubated for 2 hours at 65°C. Periodic mixing during the 2 hour incubation is required for uniform lysis of the nuclear pellet and optimum recovery of DNA. A single 1.5 ml tube can be utilized for the entire procedure. The yield is greater than 90% of theoretical with an average size greater than 300 kb. DNA concentrations are determined spectrofluorometrically with Hoechst dye (3), or by electrophoresis with a series of known DNA samples on a 0.6% agarose gel stained with ethidium bromide. Conventional UV spectroscopy cannot be used since the proteolytic digestion products are not removed from the DNA.

DNA isolated from blood is readily digestible with all restriction enzymes tested (Fig 1). The amount of restriction enzyme required for complete digestion is comparable with DNA purified by other methods. In addition to blood, DNA has been isolated from a variety of cell lines and tissues. The DNA is suitable for a number of other enzymatic modifications such as kinasing, ligation, and amplification by PCR. Utilization of this procedure allows the isolation of DNA from a large number of samples in less than 4 hours.

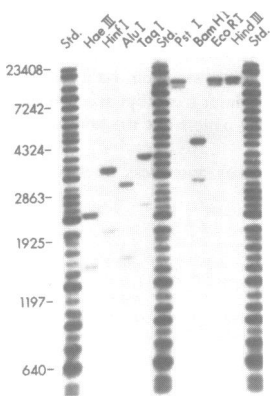

Fig. 1. Southern blot analysis of DNA from a blood sample digested with a panel of 8 restriction enzymes. Each lane contained 0.5 ug of DNA digested with 10 units of enzyme for 2 hours with the appropriate buffer. The digestion reactions were supplemented with an additional 10 mM $MgCl_2$. Following the digestion, 1/2 volume of 7.5 M $LiCl_2$ was added to each sample, mixed, and placed on ice for 10 minutes. The samples were centrifuged at 13,000 X g for 10 minutes and the supernatant transferred to a new tube. Approximately 2 volumes of room temperature absolute ethanol were added, mixed, and incubated at room temperature for 30 minutes. The DNA was pelleted, resuspended, and electrophoresed on a 1% agarose gel. Following Southern transfer, the blot was hybridized with an oligonucleotide probe to the locus D2S44. Sizes for several of the standards are indicated in base pairs on the left.

References: (1) Miller S. et al (1988) Nucl. Acids Res. 16 1215. (2) Jeanpierre M. (1987) Nucl. Acids Res. 22 9611. (3) Labarca C. and Paigen K. (1980) Anal. Biochem. 102 344-352.