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A rapid method for the purification of DNA from blood

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A simple method for DNA purification from various cell types has recently been published (1). In this protocol, cells are just dispersed in guanidine hydrochloride and DNA ethanol precipitated. Unfortunately, in my hands, the DNA was extremely difficult to resuspend when blood was used as a source. Proteinase K has been used in nucleic acid isolation for more than 15 years (2,3) and is known to be activated by chaotropic agents such as SDS or urea. This proteinase works efficiently in guanidium hydrochloride solutions and does not need to be removed from the DNA solution since proteinase K shows a marked autolytic activity at high temperature and vanishes in one hour in a 1% sarkosyl/6 M guanidine hydrochloride solution. After ethanol precipitation, DNA dissolves quickly, disclosing a causal effect of protein in difficulties in resuspension.

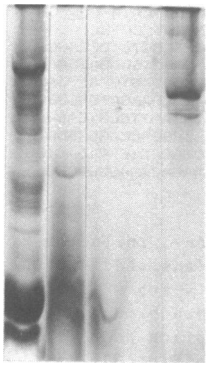

Figure 1: pattern of protein distribution in a SDS-polyacrylamide gel stained with Coomassie blue
lane 1: blood cell lysate before proteinase K; lane 2: 5 min after addition of the protease (Mw 29 500); lane 3: 1 hour after the addition of proteinase K; lane 4: DNA sample after ethanol precipitation (this protocol); lane 5: DNA sample without proteinase K; the quantity of copurified proteins varies from sample to sample.

The following protocol is now routinely used in our laboratory for preparing DNA to be used for Southern blotting (more than 100 samples have been succesfully prepared, and up to 8 restriction enzymes tested with each): 10 ml of frozen anticoagulated human blood are quickly thawed at 37°C and washed twice in 10 mM NaCl + 10 mM EDTA; the pellet (mostly cells debris and nuclei) should be almost white. This pellet is homogenized in 14 ml of filtered guanidium hydrochloride and 1 ml of 7.5 M ammonium acetate. 1 ml of 20% sodium sarkosyl and 150 µl of proteinase K (10mg/ml) are added and the mixture heated to 60°C for one hour. 35 ml of ethanol are added *at room temperature*; the DNA threads are spooled and washed twice in 70% ethanol. Excess ethanol is gently wiped off and DNA is resuspended *without any drying* in 1.5 ml of TE (10 mM Tris-HCl pH 7 + 1 mM EDTA). The mean yield is 20 µg of DNA per ml of blood.

References:
1. Bowtell, D. (1987) Anal. Biochem. 162, 463-465.
2. Wiegers, U. and Hilz, H. (1971) BBRC 44, 513-519.
3. Gross-Bellard, M. Oudet, P. and Chambon P. (1973) Eur. J. Biochem. 36, 32-38.

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