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Rapid Method for Isolation of DNA from Glass Slide Smears for PCR

Glass slide smears of cells from bone marrow aspirates or peripheral blood are widely used in pathology and hematology laboratories for routine examination of patients. Since recent reports have shown the feasibility of DNA isolation from glass slide smears or tissues [1–3], we have devised a modification of these procedures for rapid DNA extraction from the glass slide smears of bone marrow or blood and its use for polymerase chain reaction (PCR). DNA was isolated from archived glass slide smears, unstained or stained with Wright's stain. Typically, one or two glass slide smears were scraped into a sterile 1.5-ml Eppendorf tube using a new razor blade. The DNA was extracted from the resulting powder without phenol/chloroform extraction using a modification of a previously described method [4]: the scraped material was resuspended in 400 µl of 6 *M* guanidinium hydrochloride, 30 µl of 20% sodium sarcosyl, 30 µl of 7.5 *M* ammonium acetate and 10 µl of proteinase K (10 mg/ml). The mixture was heated at 60 °C for 1 h. If the powder was not completely dissolved, an additional 10 µl of proteinase K was added and the mixture was heated for another hour at 60 °C. The DNA was precipitated by addition of 1 ml of cold ethanol, gently homogenized and the mixture left at –20 °C overnight or 1 h at –70 °C. After centrifugation for 20 min the supernatant was discarded and the pellet resuspended in 50 µl of water.

An aliquot of 1–5 µl was used for each amplification by polymerase chain reaction. The reaction mix consisted of 100 µl containing 100 pmol of each primer, 200 µ*M* deoxynucleotides, 4 units of Taq Polymerase (BRL), 50 m*M* Tris HCl pH 8.3, 0.01% gelatin, 1.5 m*M* HCl and 1.5 m*M* $MgCl_2$. The reaction mix was overlaid with light-mineral oil and PCR reactions were performed in a DNA thermal cycler (Perkin Elmer Cetus). The reaction conditions consisted of initial denaturing at 94 °C for 6 min, followed by 40 cycles of 94 °C for 90 s, 55 °C for 90 s and 72 °C for 120 s, with a final polymerization step of 72 °C for 7 min [5]. We have used this procedure for amplification and dot-blot analysis by allele-specific hybridization of N-ras oncogene from glass slide smears of patients with acute leukemia with excellent results (fig. 1). The archived glass slide smears had been stored at room temperature for a period ranging from 1 month to 1 year. The suitability of DNA extracted from archived glass slide smears for the simple procedure described here may be very useful in studies of hematological disorders.

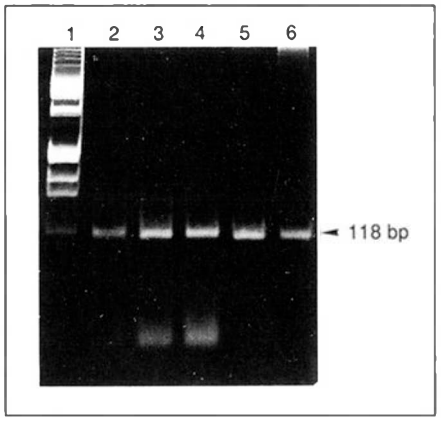

Fig. 1. Polyacrylamide gel analysis of oncogene N-ras amplification. Lane 1: ΦX 174 HaeIII, lanes 2–4: DNA extracted from glass slide smears, and lanes 5 and 6: DNA extracted from peripheral blood with phenol/chloroform.

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