

METHODOLOGY:

A simple technique for isolation of DNA suitable for PCR amplification from cytogenetic preparations

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ABSTRACT

In order to rescue molecular information from chromosome preparations, we describe a rapid procedure to obtain DNA from cytogenetic preparations in microscope slides, stored for one to five years at room temperature. This technique was modified from previously described procedures and the DNA obtained was shown to be suitable for PCR amplification.

We describe a rapid procedure to obtain DNA from cytogenetic preparations in microscope slides, stored for one to five years at room temperature. This technique was modified from previously described procedures (Jonveaux, 1991; Melo *et al.*, 1992), and the DNA obtained was shown to be suitable for PCR amplification.

Cytogenetic preparations were obtained from peripheral blood lymphocytes routinely cultivated for karyotype analysis. The cells were previously fixed in methanol: acetic acid (3:1). Some slides were also stained for sister chromatid identification (Koremborg and Freedlander, 1974).

Slides were covered with 200 µl of extraction buffer (100 mM NaCl, 50 mM Tris-HCl pH 7.5, 1 mM EDTA, 1% SDS, 1 µg proteinase K) and incubated for 30 min at 55°C, in a moisture chamber. The extraction buffer was carefully aspirated and transferred to

microcentrifuge tubes. A new aliquot of 200 µl extraction buffer was dropped onto each slide, and its surface was then scraped with a razor blade. The resulting suspensions were carefully aspirated, and added to the microcentrifuge tubes, which were incubated in a water bath at 37°C overnight. Following consecutive extractions with 400 µl of phenol: chloroform:isoamyl alcohol (25:24:1) and 400 µl chloroform:isoamyl alcohol (24:1), 300 mM sodium acetate, two volumes of cold 100% ethanol were added to the sample for chromosomal DNA precipitation. The mixture was gently homogenized and left at -20°C overnight. After centrifugation for 20 min at 12,000 rpm, the pellets were washed in 1 ml of cold 70% ethanol. The supernatant was removed and the pellets were air dried and resuspended in 20 µl of distilled water.

An aliquot of 1 µl of the extract was submitted to PCR amplification of fragments of the *NRAS* gene, codon 61. The 50 µl of reaction mix consisted of 100 pmol of the primers (5'-CAAGTGGTTATAGATGGTGA 3' and 5'-AGGAAGCCTTCGCCT 3' from Clontech, Palo Alto, California), 200 µM deoxynucleotides, 2.5 units of Taq polymerase, 50 mM Tris-HCl pH 8.3, 0.01% gelatin, 1.5 mM HCl, and 3 mM MgCl₂ (BRL, Promega). The

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reactions were performed in an automated thermal cycler (Perkin Elmer Cetus) using an initial denaturation at 94°C for 7 min, followed by 40 cycles of denaturation at 94°C for 90 sec, annealing at 55°C for 90 sec, and extension at 72°C for 2 min. A final polymerization step was done at 72°C for 7 min. After this, 5 µl of the final PCR product was run electrophoretically on a 3% agarose gel, to confirm the amplification process. Figure 1 illustrates four samples obtained through this procedure, with excellent results.

This procedure can be a useful tool to rescue molecular information from old chromosome preparations, a material that is widely available in cytogenetics laboratories.

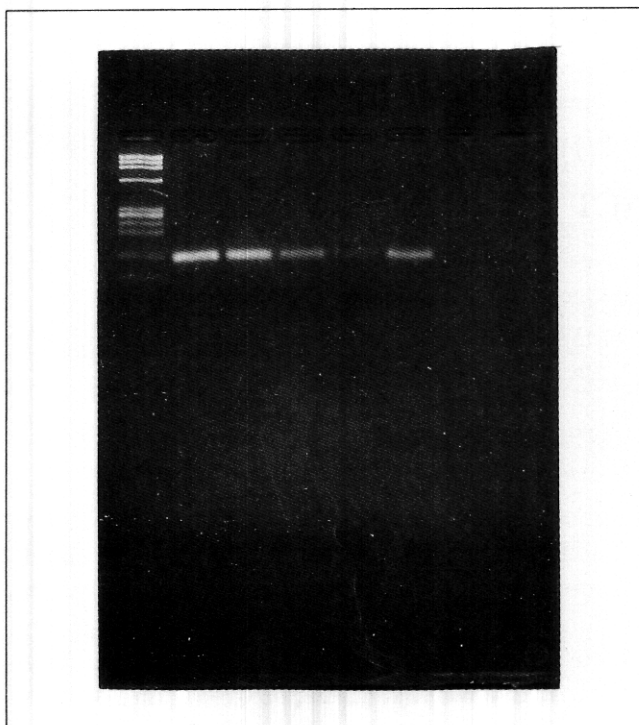


Figure 1 - Agarose 3% gel analysis of oncogene *NRAS* fragments (codon 61). From right to left, ϕ X 174 Hae III, DNA extracted from fresh leucocytes and four samples extracted from peripheral blood cultivated lymphocyte slides.

preparações citogenéticas, armazenadas por um período de um a cinco anos à temperatura ambiente. Esta técnica foi modificada de procedimentos descritos anteriormente, e o DNA obtido revelou-se adequado para amplificação pela PCR.

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(Received November 28, 1994)

ACKNOWLEDGMENTS

Publication supported by FAPESP.

RESUMO

Com o objetivo de analisar alterações moleculares a partir de preparações cromossômicas antigas, descrevemos um procedimento rápido para a obtenção de DNA de lâminas de