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DNA extraction from fixed cytogenetic cell suspensions

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ABSTRACT. We developed a procedure for DNA extraction from small volumes of fixed cell suspensions previously prepared for conventional cytogenetic analysis. Good quality DNA was isolated with a fast and simple protocol using DNAzol reagent. This provided suitable DNA for various types of molecular analyses, including polymerase chain reaction, restriction fragment length polymorphism, denaturing high-performance liquid chromatography, and direct sequencing. This technique provides sufficient material for such test, which are important for diagnosis of neoplastic diseases in pediatric patients.

Key words: DNA extraction, DNAzol, Cell suspension, Cytogenetic

INTRODUCTION

Ordinarily, cells are subjected to hypotonic treatment in cytogenetic preparations. A fixative solution (3:1 methanol:glacial acetic acid) is added to the cell suspension; usually the fixative removes lipids and denatures proteins, making the cell membrane remnants very fragile, which allows subsequent chromosome spreading and consequently exposes the DNA. These preparations are usually stored for years after chromosome analysis (Coleman and Tsongalis, 1997).

We developed a protocol for DNA extraction from fixed human cell suspensions previously prepared for cytogenetic analysis. As we have been backtracking *GATA1* mutations in newborns with Down syndrome, we tested whether frozen cell suspensions obtained for conventional cytogenetic analysis performed at birth would be suitable for molecular analyses.

MATERIAL AND METHODS

Samples

Peripheral blood and/or bone marrow cell suspensions were obtained from 30 children with Down syndrome. They were previously submitted to a karyotype analysis from 2003 to 2006 at the Genetic Service of Instituto Fernandes Figueira and Hospital Universitário Gaffreé-Guinle, Rio de Janeiro, Brazil. Samples were stored in a freezer at -20^{o}C.

The Institutional Research Ethics Committee approved the study with consent of the children's parents in all centers. We chose Down syndrome samples because there is an appropriate genetic marker and because there is indirect evidence that the leukemogenic process starts *in utero* (Gale et al., 1997).

DNA extraction

All DNA extraction and molecular analyses were performed at the Instituto Nacional de Câncer, Rio de Janeiro, Brazil. Cell suspensions were initially centrifuged to remove methanol and glacial acetic acid. These suspensions were then precipitated by centrifugation at 6000 rpm for 5 min, at room temperature. Supernatant was removed and cells were resuspended in 300 µL cold (4°C) PBS solution (NaCl, KCl, KH_2PO_4, $NaHPO_4$, $12H_2O$, pH 7.2-7.4). This washing can be repeated twice or three times to remove all methanol/glacial acetic acid residues. Cell suspensions were lysed with 1.0 mL DNAzol reagent® (Invitrogen) by gently pipetting (Chomczynski et al., 1997). A 0.5-mL aliquot of 100% ethanol was added to the DNAzol cell lysate. This mixture was shaken vigorously and stored for 5 min at -80°C. Then, centrifugations at 10,000 rpm for 5 min were performed to precipitate DNA.

After centrifugation, the supernatant was removed and 1 mL 75% ethanol was added. The tube was agitated vigorously until the DNA pellet was completely dispersed. The resulting mixture was centrifuged at 12,000 rpm for 5 min at room temperature. Ethanol wash was decanted carefully and the tubes were stored vertically for approximately 1 min. Residual ethanol was removed with a micropipette. The DNA pellet was dissolved in 50 µL 8 mM NaOH and incubated at room temperature for 3-5 min, followed by repetitive pipetting.

DNA was quantified with a NanoDrop® (ND-1000) spectrophotometer, with 1 µL of sample at 260 nm wavelength for determination of double-stranded DNA within a large dy-

namic range (2-3700 ng/µL DNA); the quality of the extraction was evaluated on an electrophoresis gel stained with ethidium bromide.

To further test the quality of the DNA, a PCR for suspension cell DNA amplification was carried out with all samples in a 20-µL reaction volume. Each reaction tube contained at least 50 ng DNA; exon 2 of the *GATA1* gene was amplified using primers described by Magalhães et al. (2006). After amplification, 5 µL PCR products was submitted to 1.5% agarose gel electrophoresis using a 100-bp ladder as a molecular weight marker. Denaturing high-performance liquid chromatography was performed on a Transgenomic WAVE System to screen for exon 2 of the *GATA1* gene.

RESULTS

We were able to isolate DNA from samples that have been collected and stored for over three years. The amount of DNA varied from 36 to 305 ng/µL, with a mean of 100.2 ng/µL (N = 30). The A260/A280 values ranged from 1.5 to 2.7. The mean value of the A260/A280 ratio was 2.1, based on Sambrook et al. (1993).

The amount and quality of DNA was suitable to amplify exon 2 of the *GATA1* gene on a 1.5% agarose gel (Figure 1).

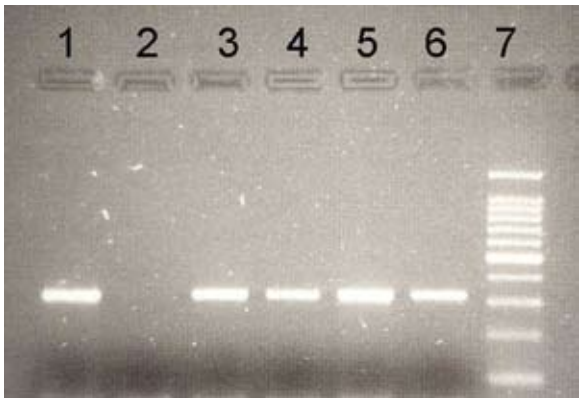

Figure 1. Exon 2 amplification of the *GATA1* gene on a 1.5% agarose gel. *Lanes 1*, *3*, *4*, *5* and *6* = DNA samples; *lane 2* = negative control; *lane 7* = 100-bp ladder.

This protocol was efficiently employed on 29 of 30 samples. PCR amplification of suspension cells taken from long-term maintenance (at least three years in storage) showed no difference when compared to short-term storage (less than one month). The amount of DNA extracted by this method was large enough to perform many PCR reactions.

DISCUSSION

In medical investigation of childhood malignancies, one of the major concerns is having enough biological material to perform important diagnostic procedures, as the mor-

tality rate is high, especially in children with congenital defects. Molecular techniques are extremely useful, particularly when the DNA can be studied at different time points along the natural history of the disease. Molecular techniques require efficient DNA extraction. One of the major limitations of PCR-based tests is inhibition of the amplification process (Coleman and Tsongalis, 1997).

The DNAzol genomic DNA isolation reagent is a ready-to-use reagent with a guanidine detergent lysing solution, which hydrolyses RNA and allows selective precipitation of DNA from a cell lysate. DNAzol has been employed for the isolation of genomic DNA from various biological materials (Chomczynski et al., 1997). Isolation of genomic DNA from fixed cells using DNAzol is fast, efficient and inexpensive. This is especially important for pediatric oncology, as the various tests require samples of peripheral blood cells and bone marrow aspirates, and under some clinical conditions the small amount of collected material limits the performance of diagnostic tests.

Although blood is the traditional source of genomic DNA, we found that cell suspensions are a viable alternative for epidemiological studies.

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