DNAzol®: A Reagent for the Rapid Isolation of Genomic DNA

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ABSTRACT

In this report, we present DNAzolÒ, a patent-pending DNA isolation reagent containing guanidine thiocyanate and a detergent mixture. It is a complete, nontoxic and ready-to-use reagent for the isolation of genomic DNA from various biological sources. In the DNAzol protocol, a biological sample is homogenized (or lysed) in DNAzol, and the DNA is precipitated with ethanol, washed and dissolved in 8 mM NaOH. Following pH adjustment, the DNA can be used immediately for analysis or stored at 4°C. The entire isolation can be completed in 20–30 min, and a wide range of DNA molecules can be isolated including genomic DNA and DNA fragments down to 0.1 kb in length. If necessary, samples can be stored in DNAzol at room temperature for extended periods of time. The isolated DNA is ready for PCR, Southern blotting and other molecular biology applications without any additional purification.

INTRODUCTION

A rapid expansion of DNA analysis in medical, biotechnological and basic research has created the need for simple and efficient commercial methods for isolating genomic DNA. Traditional DNA isolation methods based on phenol extraction, proteinase digestion or adsorption of DNA on solid matrices cannot be adapted easily to fulfill these criteria (1). DNA isolation methods with the best potential for simplicity and efficiency are methods using chaotropic agents such as guanidine salts (3,5).

In this report, we present DNAzol®, a patent-pending DNA isolation reagent containing guanidine thiocyanate and a detergent mixture. It is a complete, nontoxic and ready-to-use reagent for the isolation of genomic DNA from various biological sources. During the isolation, a biological sample is homogenized (or lysed) in DNAzol, and DNA is precipitated from the lysate with ethanol, washed and dissolved in 8 mM NaOH. Following pH adjustment, DNA can be stored at 4°C or used immediately for analysis. We describe the use of DNAzol for the isolation of genomic DNA from samples of human and animal origin. Although DNAzol can be used for the isolation of genomic DNA from plants, a modified version of DNAzol is being developed to address the unique requirements of DNA isolation from plant material.

DNAzol is available from two manufacturers: Molecular Research Center (Cincinnati, OH, USA) and Life Technologies (Gaithersburg, MD, USA).

MATERIALS AND METHODS

The DNAzol reagent was used to isolate genomic DNA according to the manufacturer's protocol. Briefly, tissue samples were homogenized in DNAzol using a glass-Teflon® homogenizer. Cells grown in monolayer were lysed by adding DNAzol directly to a culture plate. Following centrifugation of the homogenate/lysate, DNA was precipitated from the supernatant with ethanol and recovered by spooling or by a brief centrifugation. The DNA pellet was washed with ethanol and solubilized in 8 mM NaOH. If necessary, insoluble material can be removed by centrifugation. The alkaline DNA solution was adjusted to a desired pH with 0.1 M HEPES as indicated

in the manufacturer's protocol and used immediately for analysis or stored at 4°C.

Electrophoretic separation of DNA in 1% agarose and Southern analysis were performed as described earlier (4). Polymerase chain reaction (PCR) was performed using *Taq* DNA Polymerase (Perkin-Elmer, Norwalk, CT, USA) according to the manufacturer's protocol.

RESULTS AND DISCUSSION

The efficacy of DNAzol was evaluated by isolating genomic DNA from various human and animal sources. As shown in Table 1, the yield of DNA isolated from animal tissues varies from 0.9 to 7 μg DNA/mg tissue, with the exception of the spleen, yielding 22 μg DNA/mg tissue. The amount of DNA isolated from human MCF-7 and rat P0 cells was 7.1 and 6.5 μg DNA/10^6 cells, respectively. Since human cells contain 7.1 pg DNA/cell and rat cells contain 6.5 pg DNA/cell(2), the DNAzol procedure isolated > 90% of the genomic DNA from these two cell types. All preparations of DNA isolated by the DNAzol procedure had a A_{260}/A_{280} absorbance ratio of greater than 1.70 (not shown).

The efficiency of the DNAzol procedure was further evaluated in recovery experiments using high molecular weight calf thymus DNA (Sigma Chemical, St. Louis, MO, USA). DNA was dissolved in DNAzol, and the extraction procedure was performed as described in Materials and Methods. The recovery of high molecular weight DNA was 87.3% ± 8.0% (n = 3) for the isolation of 100 μg DNA and 82.2% ± 10.2 (n = 3) for the isolation of 15 μg DNA.

Table 1. Amount of DNA Isolated from Various Sources with DNAzol

Source	μg DNA[a]
Liver	4.3 ± 0.7
Kidney	3.6 ± 0.2
Spleen	22.8 ± 4.1
Heart	2.0 ± 0.4
Lung	2.5 ± 0.4
Skeletal muscle	0.9 ± 0.3
Rat P0 cells	6.7 ± 0.6
Human MCF-7 cells	7.1 ± 0.5

[a]μg DNA/mg tissue, or μg DNA/10^6 cells

Rat tissues were homogenized and cells were lysed in DNAzol as described in Materials and Methods. Results are shown as mean ± SD for 2–7 samples.

DNA isolated by the DNAzol procedure was subjected to Southern analysis and PCR. The results are shown in Figure 1. The genomic DNA isolated from rat spleen shows a typical electrophoretic pattern with about 20% of the DNA localized in proximity to the application slots and the remaining DNA

migrating as a broad 20–40-kb band (Figure 1A). The quality of the DNA isolated is evidenced by the complete restriction with *Eco*RI and by the detection of an undegraded 11.4-kb fragment of the rat growth hormone gene in a Southern analysis (Figure 1C). The isolated DNA supported PCR amplification of a 379-bp phosphoglycerate kinase gene fragment (Figure 1B). In other experiments (not shown), isolated DNA was used for amplification of various DNA fragments ranging in size from 0.2 to 7.5 kb.

The recovery of low molecular weight DNA in the DNAzol procedure was tested using λ DNA/*Hin*dIII and ϕX174 replicative form (RF) DNA *Hae*III fragments. Results presented in Figure 2 show that the DNAzol procedure efficiently isolated DNA fragments greater than 500 bp. The isolation of even smaller fragments (100–500 bp) was accomplished by performing the procedure in the presence of a carrier (Polyacryl Carrier; Molecular Research Center). The size of the isolated fragments, ranging from 23 to 0.1 kb, suggests that DNAzol can be used for the isolation of viral DNA and apototic DNA fragments.

The DNAzol procedure was used for isolating DNA from the tails of transgenic mice (Figure 3). In these experiments,

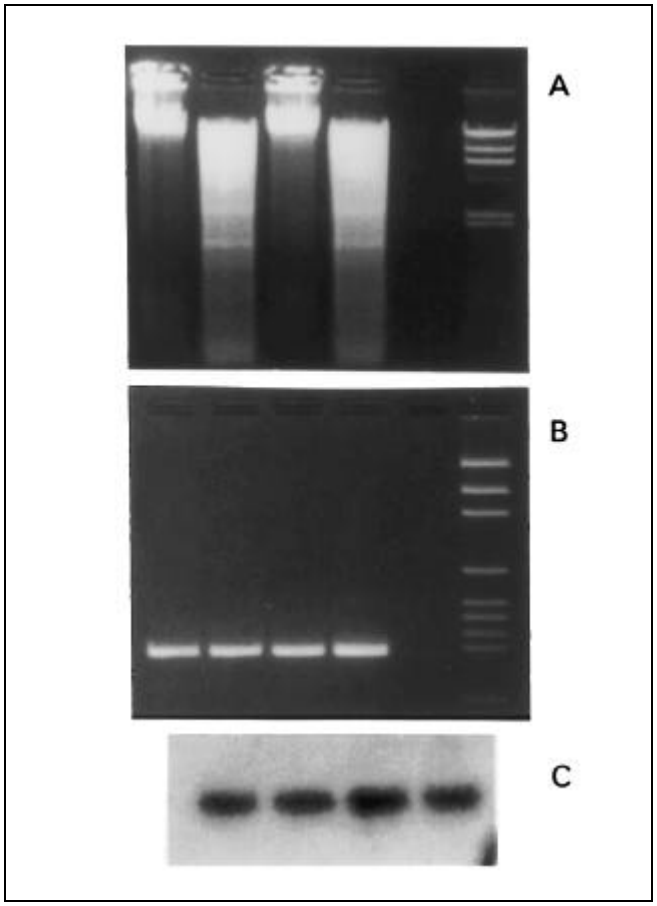

Figure 1. Analysis of DNA isolated from rat spleen using DNAzol. Each panel shows results obtained with two DNA preparations. A) DNA (3 µg) was electrophoresed in 1% agarose gel with ethidium bromide. Shown is intact DNA and DNA digested with *Eco*RI for 18 h at 37°C. Last lane shows λDNA/*Hin*dIII molecular weight markers. B) Product of PCR performed with 50 ng of DNA template. PCR mixture contained 100 ng of primers for a 379-bp phosphoglycerate kinase gene fragment. C) Detection of an 11.4-kb fragment of the rat growth hormone gene by Southern analysis. Genomic DNA (5 µg) was restricted with *Eco*RI and analyzed for the presence of the growth hormone gene using nick-translated growth hormone cDNA.

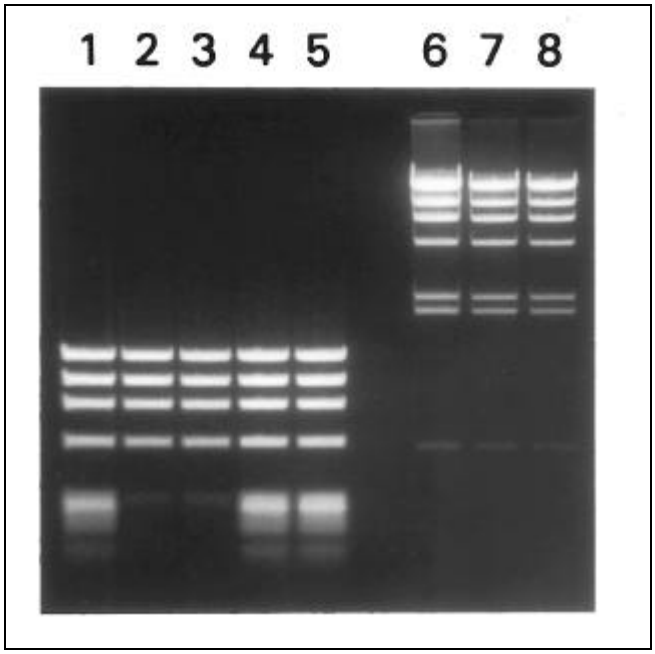

Figure 2. Electrophoretic separation of DNA fragments isolated with DNAzol. ϕX174 RF DNA/*Hae*III fragments (8 µg) and λDNA/*Hin*dIII fragments (8 µg) were isolated using DNAzol and electrophoresed in 1% agarose-ethidium bromide gel. ϕX174 RF DNA fragments before isolation, lane 1; after isolation, lanes 2 and 3; and after isolation in the presence of Polyacryl Carrier, lanes 4 and 5. λDNA fragments before isolation, lane 6; and after isolation, lanes 7 and 8.

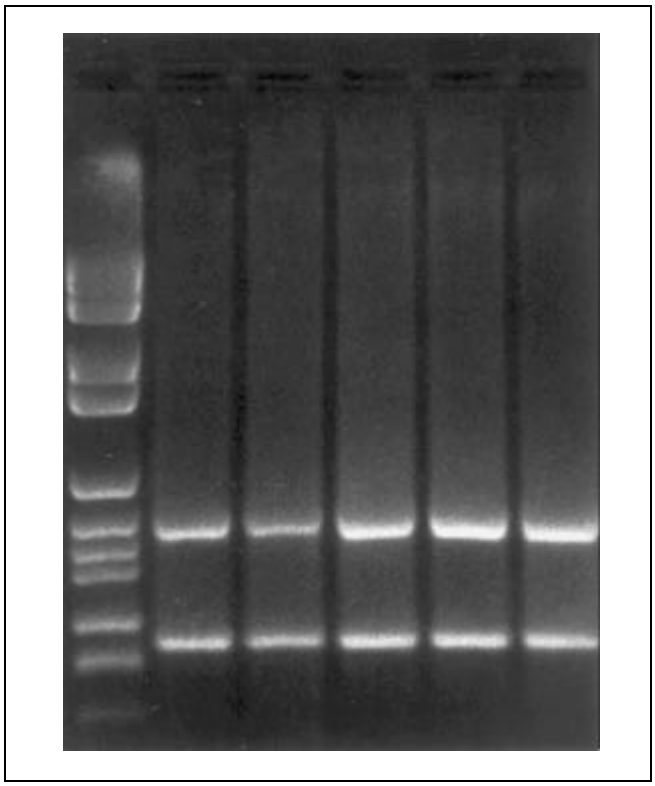

Figure 3. PCR amplification of DNA isolated from transgenic mouse tails with DNAzol. Mouse tails (20–40 mg) were minced and incubated in 1 mL DNAzol supplemented with 0.4 mg of proteinase K. Following overnight incubation, genomic DNA was isolated as described in Materials and Methods. PCR was performed simultaneously with two primer pairs: for a 502-bp fragment of the human protein C transgene and a 216-bp fragment of the endogenous mouse whey acidic protein. Shown are results of isolation from five transgenic mice.

tail fragments were minced, incubated overnight with DNAzol supplemented with proteinase K and processed with DNAzol (without homogenization) as described in Materials and Methods. The yield was 0.4–2 μg DNA per milligram of tail tissue, with the A_{260}/A_{280} ratio of the isolated DNA ranging from 1.77 to 1.92.

In addition, the DNAzol procedure was used for the isolation of genomic DNA from whole blood, yielding about 35 μg of DNA per milliliter of human blood.

Experiments were performed to investigate long-term storage of samples in DNAzol. Following homogenization, samples were stored in DNAzol for one month at room temperature and for ten months at 4°C. Neither PCR nor the electrophoretic pattern of DNA was affected by the long-term storage of samples in DNAzol (results not shown).

In summary, the DNAzol reagent described here offers a fast and effective method for the isolation of DNA from tissue and cell samples. The isolation protocol is simple and it can be completed in 20–30 min. If necessary, samples can be stored in DNAzol at room temperature for extended periods of time. Consequently, DNAzol can be used to collect DNA samples in the field without the necessity for refrigeration of the collected samples. By using DNAzol, a wide range of DNA molecules can be isolated including genomic DNA and DNA fragments down to 0.1 kb in length. The isolated DNA is ready for PCR, Southern blotting and other molecular biology applications without additional purification.

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