DNA Isolation by a Rapid Method from Human Blood Samples: Effects of MgCl₂, EDTA, Storage Time, and Temperature on DNA Yield and Quality

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The isolation of DNA from whole blood by a modified rapid method (RM) was tested using various detergents and buffer conditions. Extraction of DNA with either NP-40 or Triton X-100 gave a high yield of undegraded DNA in less than an hour. The concentration of magnesium ion in the buffers was critical to obtaining intact, high molecular weight (HMW) DNA. Greater than 10 mM MgCl₂ led to degradation. Addition of EDTA to the buffer inhibits this degradation. Preparation of DNA from blood stored at room temperature or incubated at 37°C for 24 hr resulted in the same amount and quality of DNA as from samples frozen at −70°C. DNA from blood samples that had undergone more than four freeze-thaw cycles was found to be partially degraded. The modified RM can be applied to extract DNA from as little as 10 µl of blood (340 ng of DNA) and from dried blood samples. DNA samples remained intact and undegraded for longer times when DNA was dissolved in higher concentrations of EDTA.

KEY WORDS: high molecular weight DNA; MgCl₂; integrity of DNA; rapid method; DNA banking.

INTRODUCTION

The application of genetics to study human disease (Anderson, 1992; Miller, 1992) and to analyze gene function in vivo depends upon the quality of DNA samples. Intact and good-quality DNA is also essential for screening DNA

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samples to study polymorphisms. However, these studies often require analysis of a large number of DNA samples, which must be of sufficient quality for Southern blot analysis. Recently, a fast, safe, and economical method (RM) was developed to extract DNA from whole blood (Lahiri and Nurnberger, 1991). The main advantage of the RM is that it is a nonenzymatic and inorganic procedure. There are two important aspects of DNA preparation: yield, and degree of degradation of DNA samples. The present report deals with various factors that influence the integrity of DNA. We show that the concentration of MgCl$_2$ is crucial to obtain intact, high-yield DNA from whole blood. We present a simplified version of the RM that extracts DNA with fewer steps and with less manipulation. We have examined the effect of blood storage time and temperature on DNA yield and quality and have studied the state of extracted DNA in different buffers.

**MATERIALS AND METHODS**

Brij-35, digitonin, nonidet P-40 (NP-40), Sarcosyl, Triton X-100, Tween-20, and Tween-80 were bought from Sigma Chemical Co. Other chemicals used were analytical grade (Sigma). Whole blood was collected in a Vacutainer tube (purple-stoppered) containing 100 µl of 15% EDTA. The simplified version of the RM is described below. One milliliter of blood was treated with an equal volume of low-salt buffer containing 10 mM Tris–HCl, pH 7.6, 10 mM KCl, 2 mM EDTA (TKE) containing 4 mM MgCl$_2$ (TKM). Twenty five microliters of NP-40 was added and the cells were lysed by inverting several times. The suspension was centrifuged at 1000g for 10 min at room temperature (RT). The pellet of mostly leukocytes was saved and washed two more times with TKM buffer. The final pellet was resuspended in 0.2 ml of TKM buffer. Fifteen microliters of 10% sodium dodecyl sulfate (SDS) was added, and the whole suspension mixed thoroughly and incubated for 5 min at 55°C. After adding 75 µl of saturated NaCl (~6 M), the tube was mixed well and centrifuged at 12,000g for 5 min. The supernatant contained DNA, which was precipitated using ethanol. DNA was redissolved in 0.5 ml of 10 mM Tris–HCl, 1 mM EDTA, pH 8.0 (TE). The yields of DNA were calculated from the absorbance at 260 nm (Maniatis et al., 1982) and by comparison with a molecular weight DNA standard on an agarose gel stained with ethidium bromide.

**RESULTS**

*Effect of Different Detergents in the Extraction of DNA from Blood Samples*

Two kinds of detergents were used to study their effects in the extraction of DNA. One group lysed the red cells, resulting in a clear solution; the other