SHORT COMMUNICATION

Isolation of Restrictible DNA

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Summary: A simple method for the isolation of pure and high-yield DNA from whole blood, suitable for restriction enzyme digestion, is described. The steps of the procedure are as follows: cell lysis with \( \text{NH}_4\text{Cl}, \text{NaHCO}_3, \text{EDTA} \); digestion with proteinase K in the presence of SDS; extraction with phenol-chloroform-isoamyl alcohol; and precipitation with ethanol. The 260 nm/280 nm absorbance ratio showed a mean value of 2, and the average yield of DNA was 212 \( \mu \text{g/l} \). Such DNA preparations were found to be quite suitable for digestion by a variety of restriction endonucleases, as well as for the analysis of gene disorders by different biological methods. The method proposed appears to be useful in clinical chemistry laboratories.

Introduction

Molecular biotechnological studies on gene structure and DNA restriction fragment analysis require the isolation of genomic DNA in a form that can be cleaved by restriction enzymes, i.e. purified and intact DNA which will not produce non-specific DNA fragments by casual splitting. Great variability in the digestion by restriction enzymes has been attributed to the source of DNA and the method of its preparation. A series of procedures for DNA isolation have been described; these differ in some seemingly petty details, which are, however, very important for both the quality and quantity of isolated DNA.

The aim of this study was to isolate a restrictible DNA, i.e. an adequately pure and intact DNA for Southern blot analysis or for the analysis of restriction fragments from whole blood samples (1—5).

Materials and Methods

Chemicals, solutions

Erythrocyte lysis solution: 150 mmol/l \( \text{NH}_4\text{Cl}, 10 \text{ mmol/l NaHCO}_3, 0.1 \text{ mmol/l EDTA} \) (Kemika, Zagreb)

Buffer for leukocyte suspension: 100 mmol/l \( \text{NaCl}, 50 \text{ mmol/l TRIS, 1 mmol/l EDTA} \) (Kemika, Zagreb), pH 7.4

SDS: 100 g/l (BioRad, Milan)

Proteinase K: 10 g/l (Boehringer Mannheim)

Phenol S: unoxidized phenol, extracted twice with 1 mol/l TRIS (pH 8.0), with added 8-hydroxyquinoline (final concentration, 1 g/l), 1 × extracted with 0.1 mol/l TRIS containing mercaptoethanol (final concentration 1 g/l) (Merck, Darmstadt)

Phenol/chloroform (1 + 1)

Chloroform I: chloroform and isoamyl alcohol (24 + 1)

Absolute alcohol (Kemika, Zagreb)

Ethanol: volume fraction 0.70 (Kemika, Zagreb)

TRIS/EDTA buffer: 10 mmol/l TRIS-HCl, pH 7.5; 1 mmol/l EDTA

All reagents were prepared as stock solutions and sterilized by filtration or autoclaving (6).

Procedure of isolation

Ten ml of whole blood were mixed with four volumes of red cell lysis buffer in disposable plastic 50 ml tubes (Nunc, Roskilde, DK) and placed in an ice water mix for 10 min at \(+4\) °C. The supernatant was discarded and the white cell pellet resuspended in lysis buffer until freeing it from any red cell membrane debris (usually twice). The purified white cell pellet was resuspended in 10 ml white cell suspension medium, to which 1 ml 100 g/l SDS (final concentration, 10 g/l) was added, and mixed gently to lyse the cells; then, 50 \( \mu \)l Proteinase K were added and incubated in a water bath at 37 °C overnight. On the next day, the sample was observed to have become viscous. DNA liberated from white cells was gently but thoroughly extracted with an equal volume of phenol for 10 min at \(+4\) °C. The supernatant was discarded and the white cell pellet resuspended in lysis buffer until freeing it from any red cell membrane debris (usually twice). The purified white cell pellet was resuspended in 10 ml white cell suspension medium, to which 1 ml 100 g/l SDS (final concentration, 10 g/l) was added, and mixed gently to lyse the cells; then, 50 \( \mu \)l Proteinase K were added and incubated in a water bath at 37 °C overnight. On the next day, the sample was observed to have become viscous. DNA liberated from white cells was gently but thoroughly extracted with an equal volume of phenol for 10 min, resulting in a homogeneous mixture. Shorter periods are insufficient for adequate extraction of proteins. The phases were separated by centrifugation at 2500 \( \times \) g at \(+4\) °C for 10 min. Dissolved DNA in the upper aqueous layer was removed with a wide bore pipette to a fresh 50-ml tube. The separation was very carefully performed to transfer of the protein remnants from the contact layer between the aqueous phase and phenol/chloroform. In a pooled aqueous layer, DNA was extracted with an equal volume of chloroform/isoamyl alcohol (24:1). The aqueous layer was carefully separated into 150 ml Erlenmeyer flask (without protein debris or phenol-chloroform drops). DNA was precipitated by the addition of 4 volumes of absolute alcohol, which was
1. Add lysis buffer, mix on ice, centrifuge

2. Add leukocyte suspension buffer, mix
3. Add SDS, mix
4. Add proteinase K

5. Extract with phenol/chloroform and centrifuge (twice)

6. Extract upper layer with chloroform and isoamyl alcohol mixture, centrifuge

7. Transfer upper layer, add absolute alcohol, rotate gently

8. Transfer to ethanol (volume fraction 0.70), wash, dry in air

9. Add Tris/EDTA buffer, mix, store at +4 °C

Fig. 1. Procedure of DNA purification from whole blood.

To compare the proposed method (A), a high molecular genomic DNA was prepared from whole blood by the methods of Walker (7) and Boulnois (8), designated as methods B and C, respectively.

Recovery of DNA was measured by two criteria:

(a) UV absorbance ratio (6); and
(b) minigel electrophoresis (9).

Samples containing 5 µg of DNA were digested by different restriction endonucleases (Hind III, Bam HI, and Eco RI, Boehringer Mannheim) in a 50-μl reaction volume according to the regulations for the action of restriction enzymes and for DNA standards. The minigel electrophoresis of the split DNA was performed on 10 g/l agarose dissolved in TRIS-acetate buffer, pH 8.3, at 3 V/cm for 90 min. The gel was stained with ethidium bromide, illuminated with UV and photographed with Kodak film.
Results

DNAs were isolated from the total of 11 samples using the methods described above. Results on the purity and yield of various isolation procedures of DNA are shown in Table 1.

DNAs isolated by all three methods from fresh material, frozen leukocytes and frozen DNA, and tested by minigel electrophoresis are presented in Figure 2. As can be seen, the DNA molecule isolated by method A moved as a single band from the very start, and so did the DNA molecules isolated by methods B and C, suggesting that the procedure of isolation did not cause any non-specific digestion, although a thin smear could be observed on lanes 1 and 2 of DNA prepared by methods B and C. The stability of DNA in blood samples stabilized with EDTA as an anticoagulant was investigated by purifying DNA from the samples stored at -20 °C for 2 months. The electrophoretic pattern of these results shows degradation of DNA, even in blood stored at -20 °C. DNA purified from fresh material and subsequently stored at -20 °C for 2 months (lanes 6, 7 and 8) underwent no degradation under these conditions. Lanes 10, 11 and 12 show the pattern of restrictibility of DNA purified by method A from fresh leukocytes and digested with restriction endonucleases Hind III, Eco RI and Bam HI for 1 hour at 37 °C.

Discussion

Genomic DNA from human whole blood can be purified by several techniques involving a variety of detergents and denaturating agents, but each of these methods shows some advantages and disadvantages (10–13). In general, the procedure consists of two parts: a technique of gentle lysis of the cells and DNA solubilization, followed by one of several basic enzymatic or chemical methods to remove contaminating proteins, RNA and other macromolecules. In our attempts to find a suitable method which will ensure intact and restrictible DNA in an amount sufficient for different analyses at the molecular level, we slightly modified the methods of Walker (B) and Boulois (C). From Walker's method, the step of blood homogenization was omitted, and in the case of Boulois' method, proteinase K for protein digestion was added. Also, a greater amount of buffer (10 ml) for leukocyte suspension was added in our method to obtain a less viscous sample, thus allowing a more homogeneous phenol extraction step and better yield of DNA. By these slight modifications, a somewhat simpler procedure of isolation was established, with quite a good and reproducible yield of DNA.

Investigation of the DNA by minigel electrophoresis showed approximately identical patterns of DNA, suggesting that iso-

Tab. 1. Purity and yield of various DNA isolation procedures compared*.

<table>
<thead>
<tr>
<th>Isolation procedure</th>
<th>Absorbance ratio</th>
<th>Yield</th>
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<tr>
<td></td>
<td>260 nm/280 nm</td>
<td>g/l</td>
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<tr>
<td>Proposed method</td>
<td>( \bar{x} = 2.0 ) range (1.95–2.10)</td>
<td>( \bar{x} = 0.212 ) range (0.170–0.260)</td>
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<tr>
<td>Walker's method</td>
<td>( \bar{x} = 2.0 ) range (1.95–2.05)</td>
<td>( \bar{x} = 0.160 ) range (0.56–0.360)</td>
</tr>
<tr>
<td>Boulois' method</td>
<td>( \bar{x} = 1.8 ) range (1.7–1.8)</td>
<td>( \bar{x} = 0.213 ) range (0.210–0.216)</td>
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* Results are presented as mean and range of absorbance ratio, and DNA concentrations of 11 blood samples from the same blood pool extracted in a single run (12).
lation by any of the methods did not cause non-specific digestion. Frozen leukocytes (−20 °C/2 months) are a less suitable material for isolation and purification of restrictible DNA because of DNA degradation. At the same time, the DNA purified from a fresh sample and stored at −20 °C did not show any degradation after two months and was suitable for digestion with restriction enzymes. Comparison of the restrictibility of purified DNAs indicated that the best electrophoretic pattern was obtained with DNA isolated and purified by our method.

In conclusion, the DNA obtained by the procedure described above appears to be essentially pure and suitable for numerous applications. The average yield (0.212 g/l of whole blood) is sufficient for many gene structure analyses, using Southern blot analysis and restriction fragment length polymorphism studies, either with or without restriction analysis. By gel processing and drying following electrophoresis, the fluorescent colour remains on electrophoretic bands, so that such a minigel plate requires no reading-off immediately after the completion of electrophoresis with an UV lamp. Thus, the method appears to be absolutely appropriate for work in any clinical laboratory.

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References


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