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Purification of genomic DNA from human whole blood by isopropanol-fractionation with concentrated NaI and SDS

Lu Wang, Kazunari Hirayasu*, Masaki Ishizawa and Yoshiteru Kobayashi
Osaka Research Laboratories, Wako Pure Chemical Industries, Ltd, 6-1, Takada-cho, Amagasaki, Hyogo 661, Japan

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Many applications of gene analysis call for rapid and efficient methods for isolation of genome DNA from whole blood and complex biological samples. Relating to these demands, many protocols have been published (1–6). However, either the methods involves toxic organic extractions and/or skillful manipulations such as spooling of DNA with an inoculation loop and the like to separate DNA from the other cellular compounds, and little was discussed about recovery of DNA from cells. Here, we describe a simple and efficient method utilizing fractionation of DNA with isopropanol from nuclear lysate in the presence of concentrated NaI and SDS, a modified technique of that previously reported in isolation of nucleic acid from serum (7). By use of SDS instead of sodium *N*-laurylsarcosinate, the technique was largely improved in separation of DNA, leaving other cellular materials in the solution. The purification took about 1.5 hr and brief differential centrifugation followed by decantation was done several times with a single tube with cap at room temperature. The method overcome such the disadvantages as were seen in existing methods and realized a high yield of DNA. Therefore, it would be usable for simultaneous isolation of genomic DNA from large numbers of samples of whole blood.

The method is summarized in Fig. 1 as the standard protocol. First, 0.5 ml of human whole blood anticoagulated with EDTA-K_2 at 1 mg/ml blood was mixed with an equal volume of a lysis solution containing 1% Triton X-100 to lyse the cells and the nuclei were isolated as described (8). Isolated nuclei were suspended in an enzyme reaction solution containing 1% SDS and digested with 0.8 mg/ml proteinase K to liberate DNA from nuclear proteins. After 1 hr-incubation, NaI solution was added to the nuclear lysate to the final concentrations of 4.5 M NaI and 0.4% SDS, and was followed by isopropanol addition. The content in the tube was mixed well by inversion until whitish precipitate appeared. The precipitate was collected by centrifugation and washed with the alcohol solutions. If required, contaminant RNA can be removed by pancreatic RNAse treatment prior to the proteolysis.

We tried the method with different volumes of a blood specimen in the range of 0.1–1.0 ml by varying volumes of the working solutions in Fig. 1 proportional to the sample volume. The method was reproducible in both yield and purity among different sample volumes examined, which is shown in Table I.

Table II shows the results obtained by the standard method from five human blood specimens. The yield of DNA obtained by the method was comparable to that estimated by the leukocyte number visually counted with hemacytometer (9), and was much higher than that obtained by the classic method involving phenol extractions (2), of which the value was shown in the parenthesis in Table II as a comparison. The averaged recovery with the

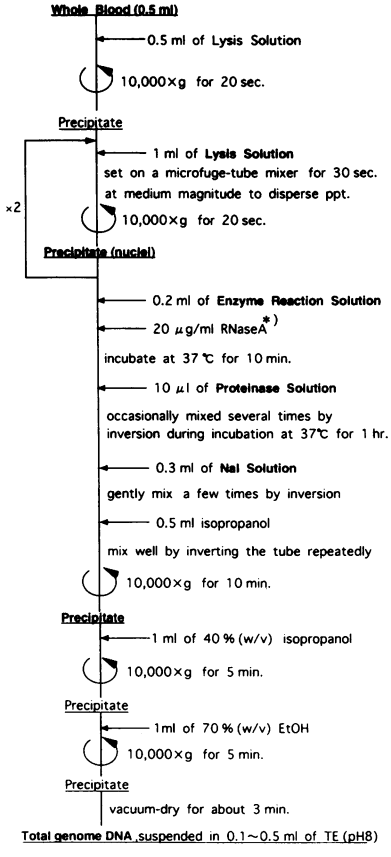

Figure 1. The Standard Protocol for Purification of Genomic DNA from Human Whole Blood. The working solutions are as follows. **Lysis Solution**: 1% (w/v) Triton X-100, 0.32 M saccharose, 5 mM $MgCl_2$, 10 mM Tris–HCl (pH 7.5); Enzyme **Reaction Solution**: 1% (w/v) SDS, 5 mM EDTA–Na_2, 10 mM Tris–HCl (pH 8.0) (8); **Proteinase Solution**: 17 mg/ml proteinase K; **NaI Solution**: 7.6 M NaI, 20 mM EDTA–Na_2, 40 mM Tris–HCl (pH 8.0). *This step is usually omitted. If tried, a concentrated RNAse A solution was added to the nuclear lysate.

* To whom correspondence should be addressed

Table I. Reproducibility of the method with different sample volumes[a]

Sample Volume (ml)	Yield of DNA (μg/ml blood)	Purity (A_{260}/A_{280})
0.1	23.8	1.97
0.5	22.0	1.92
1.0	23.0	1.90

[a]DNA was extracted from indicated sample volume of a blood specimen by the method with the working solutions at the proportion to the sample volume Fig. 1. Yield was calculated with the absorbance of 260 nm as 1 mg/ml DNA equal to A_{260} of 20.

Table II. Yield and purity of genomic DNA purified from human whole blood by the standard method and by the phenol method@

Specimen	Yield[b] (μg/ml blood)	Estimated Yield[c] (μg/ml blood)	Recovery[d] (%)	Purity (A_{260}/A_{280})
1	54.2 (31.0)	45.0	120 (69)	1.86
2	49.2 (31.8)	59.4	83 (38)	1.92
3	71.6 (60.6)	62.4	115 (97)	1.94
4	51.3	54.0	95	1.96
5	22.0	22.2	99	1.92
5 (2hr)	22.2	22.2	100	1.89
5 (24hr)	22.4	22.2	101	1.90

[a]Genomic DNA was isolated from five human blood specimens by the standard method in Fig. 1 or by the phenol method of Blin and Stafford (1) with modification (2) using 0.5 ml aliquot of the blood. In addition, the aliquots of specimen 5 were tried to isolate genomic DNA by the method with proteolytic digestion times indicated. The values obtained by the phenol method were shown in the parentheses. [b]Yield was calculated by the absorbance of 260 nm as 1 mg/ml DNA equal to OD_{260} of 20. [c]Yield was estimated by the leukocyte number with an assumption that a leukocyte contains diploid DNA of 6 pg. The cell number was determined by the cells manually counted with Burker-Turk hemacytometer with Unopette algorithm (9). The CV% is usually ±10%. [d]Recovery was expressed by the percentage of the yield with the standard method or with the phenol method (shown in parentheses) relative to that of genome DNA estimated with the leukocyte number.

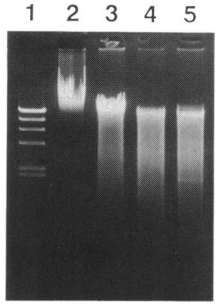

Figure 2. Electrophoresis of Genomic DNA Purified from Human Whole Blood by the Standard Method and of its Digests with Restriction Endonucleases. Lane 1 contains λ-HindIII digest. Lane 2 contains 0.5 μg of genomic DNA; lanes 3–5 contain its digests with BamHI, EcoRI, and HindIII, respectively. The genomic DNA was purified from human whole blood by the standard method. Isolated DNA was digested with respective restriction enzyme at 1–3 unit(s)/μg DNA for 3 hr at 37°C in the reaction buffer supplied by the manufacturer (Nippon Gene Co. Ltd, Toyama, Japan), and analyzed on an 1% agarose gel in TAE buffer containing EtBr. DNA was visualized with uv light.

method was 102 ± 13%, mean ± SD, whereas, the recoveries with the classic method shown in the parentheses were lower and divergently varied among different specimens. The purity

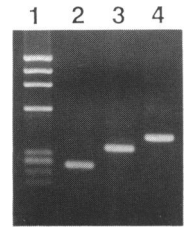

Figure 3. Electrophoresis of PCR-Amplified Products within the Human β-globin gene. Lane 1 contains φX174–HaeIII digest. Lanes 2–4 contain amplified products; 262, 345, and 408 bp, resulted from use of primer pairs; KM29/KM38, KM29/GH21, and GH20/GH21 (Takara Shuzo Co. Ltd, Kyoto, Japan), respectively. The PCR-amplifications with 30 thermal cycles for the regions within the gene were done as described elsewhere (7, 10) using 1 μg of isolated DNA from human whole blood by the standard method as a template. Of 100 μl of the reaction mixture, 2 μl was analyzed on a 2% agarose gel in TAE buffer containing EtBr. DNA was visualized with uv light.

indicated by the absorbance ratio was consistent with those reported by the others (2, 5). The indexes averaged 1.92 ± 0.03. Unlike as reported (5) with other methods (3, 4), prolong digestion of the nuclei with proteinase K more than 1 hr hardly increased the yield, indicating DNA was maximally liberated from nuclear matrix under the conditions used. All these results suggest that by this method, genomic DNA is recovered with less little loss compared with the other methods.

For biochemical properties, the DNA preparation migrated in the area above 23 K bp DNA marker on 1% agarose gel analysis, indicating the DNA maintains desirable high-molecular-weight, as shown by lane 2 in Fig. 2, and was pure enough for use as a substrate for restriction endonucleases (Fig. 2) or as a template for PCR-amplification (10), shown in Fig. 3.

Finally, the standard protocol was applicable to isolation of genomic DNA from a few cell lines although RNAse treatment was necessary to eliminate contaminant RNA (data not shown). The method is done with a single tube, which might minimize loss and fragmentation of DNA as is caused by transfer of DNA from one tube to the other being involved in the other methods. It would be widely used because of its simplicity, efficiency and versatility.

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