

A SIMPLE METHOD FOR EXTRACTION AND PURIFICATION OF GENOMIC DNA FROM DRIED BLOOD SPOTS ON FILTER PAPER

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Abstract. We have developed an efficient and simple method for extracting and purifying genomic DNA from dried blood stored on filter paper. The quality of the genomic DNA extracted is tested by PCR amplification of a 255-bp fragment of the PAX8 gene sequence and the PCR products are determined for further genetic studies by single strand conformation polymorphism (SSCP) analysis. Larger DNA sequences of the 674-bp of the PAX8 gene and the 1,039-bp of the human β -globin gene, a housekeeping gene, have also been amplified from the extracted DNA, thus indicating the high quality of the genomic DNA extracted by the developed method for subsequent genetic studies of any gene of interest. The method developed can also be used for the purification of genomic DNA from dried blood specimens stored under different conditions. Moreover, the genomic DNA products can be stored for long-term use due to the highly purified procedure. Therefore, the method is efficient and appropriate for the extraction and purification of genomic DNA from dried blood specimens, which has become an increasingly important tool for genetic and epidemiological studies.

INTRODUCTION

Collection of blood onto filter paper has become a significant tool for screening individuals for clinical purposes, *ie* mass neonatal screening for inborn diseases, and for epidemiological studies by determination of biological markers, *eg* serodiagnosis for HIV infection (Panteleeff *et al*, 1999) or hepatitis C infection (Parker *et al*, 1999). This type of specimen has been used for public health purposes since the 1960s (Guthrie and Susi, 1963) and has become increasingly popular due to its ease and convenience in collection and transportation, even from geographically isolated populations. Mei and colleagues (2001) summarized a partial list of numerous analyses that have been measured from dried blood spot specimens. Generally, any biological markers that can be measured from whole blood, serum or plasma can be determined from dried blood specimens (Mei *et al*, 2001). That includes DNA, which is im-

portant for research or studies in genetics. However, an efficient method is required to recover DNA from dried blood specimens. Commercially available kits for extracting DNA from dried blood samples are expensive and involve spin columns which provide low recovery rates. Moreover, the spin column kits always result in increased plastic waste.

We therefore developed a simple but efficient method for extracting and purifying genomic DNA from dried blood specimens. We showed that this method is sensitive and appropriate for determining genomic DNA for subsequent genetic studies.

MATERIALS AND METHODS

Dried blood spots, obtained from volunteers after obtaining written consent, were collected on filter paper (Schleicher & Schuell, Keene NH, USA) according to National Committee on Clinical Laboratory Standards' (NCCLS) specifications (Hannon *et al*, 1997). Three different sets of samples were kept at 4°C, -20°C and room temperature for about 2 weeks until experimentation.

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The simple method developed for the efficient extraction and purification of the genomic DNA from dried blood spots is as follows. Three pieces of 3-mm diameter circle were punched out from the dried blood spot samples into a 1.5-ml microcentrifuge tube. Rehydration was performed by adding 300 μ l of a 155 mM NH_4Cl (Merck, Darmstadt, Germany), 1 mM KHCO_3 (Merck) and 0.01 mM EDTA (Fluka, Neu-Ulm, Switzerland) solution with vigorous vortexing for 1-2 minutes. The mixture was incubated at room temperature for 5 minutes with subsequent removal of the supernatant. A mixture of 160 μ l double distilled water, 200 μ l of 2X proteinase K buffer [300mM NaCl (Merck), 40mM EDTA, 20mM Tris (Fluka) pH 7.5, 2.5% sodium dodecyl sulfate (ICN Biochemical Inc, OH, USA)] and 40 μ l of proteinase K (Boehringer Mannheim GmbH, Mannheim, Germany) (20 mg/ ml) was then added to the remaining blood spot circles followed by vortexing for 20 seconds. The mixture was incubated at 56°C for 1 hour. During the incubation, the mixture was resuspended by vortexing for 20 seconds every 15 minutes. Genomic DNA was then extracted from the mixture using 200 μ l of buffered phenol (USB Corporation, OH, USA) and 200 μ l of chloroform (Merck) and isoamyl alcohol (Merck) mixture (24:1), with subsequent vortexing for 30 seconds. The mixture was centrifuged at 10,000 rpm for 4 minutes at room temperature. The aqueous phase was transferred to a fresh microcentrifuge tube and added with 40 ml of 3.0 M sodium acetate (Sigma Chemical Co, MO, USA) pH 5.2 and 400 ml isopropyl alcohol (Merck). The solution was mixed and centrifuged at 10,000 rpm, for 4 minutes at room temperature. The supernatant was removed and the pellet was washed with 70% ethanol. For long-term use, the pellet could be stored in 70% ethanol at -20°C. Prior to use, the pellet was centrifuged to remove the washing. The pellet was dried at room temperature until there was no trace of ethanol and the genomic DNA was resuspended in 50 μ l of TE buffer [10 mM Tris (USB Corporation), 1 mM EDTA].

PCR analyses for amplification of small, medium and large DNA fragments

The quality of genomic DNA extracted by this method was determined by PCR amplifica-

tion of the fragment of the PAX8 gene (Strachan and Read, 1994) using primers corresponding to intron sequences flanking of the exon 5 of the PAX8 gene: the upstream primer 5' TCTCCC TCTCCCCCACTG 3' and the downstream primer 5' GCAGAGCCCCTACAAAGTCC 3'. The PCR reaction was carried out in 50 μ l of total volume containing 1 μ l of the genomic DNA extract, 0.2 μ M of each primer, 40 μ M of each deoxyribonucleotide (Boehringer Mannheim GmbH) and 1.0 unit of Taq DNA polymerase (Qiagen GmbH, Hilden, Germany) using the Perkin Elmer 9600 PCR machine. For positive control, 200 ng of genomic DNA extracted from fresh blood was used as a template. The amplifying reactions consisted of one cycle of template denaturation at 94°C for 2 minutes, 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 70°C for 30 seconds, followed by one cycle of 70°C for 5 minutes. Five microliters of the PCR product of 255 bp was visualized following electrophoresis on an ethidium bromide stained 1% agarose gel compared with 100 bp DNA ladder (New England Biolabs, MA, USA).

To confirm the genomic DNA quality, PCR analyses to amplify larger fragments of the PAX8 and β -globin genes of 674 bp and 1,039 bp, respectively, were performed. The PCR reactions were performed using similar conditions to those described above but with different primer sets and with a slightly different temperature cycling profile. To amplify the 674-bp PAX8 sequence, the upstream and downstream primers, 5' TCTCCC TCTCCCCCACTG 3' and 5' CACAGGCTCA TTTGGAGAAT 3' respectively, were used with the following cycling profile: one cycle of template denaturation at 94°C for 2 minutes, 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute, followed by one cycle of 72°C for 5 minutes. To amplify the 1,039 bp fragment of the β -globin gene, the upstream primer 5' TGCCTA TTGGTCTATTTTCC 3' and downstream primer 5' AATCCAGCCTTATCCCAACC 3' were used. The cycling profile was as followed: one cycle of template denaturation at 94°C for 2 minutes, 40 cycles of denaturation at 94°C for 30 seconds, and annealing and extension at 60°C for 1 minute 30

seconds, followed by one cycle of 70°C for 5 minutes. The PCR product was analyzed by agarose gel electrophoresis, using the same conditions mentioned above.

Single strand conformation polymorphism (SSCP)

The 255-bp PCR product of the PAX8 gene was further analyzed by an SSCP analysis, one of the efficient techniques for screening genetic variations, even a single mutation (Orita *et al*, 1989), following a procedure described in a previous report (Akkarapatumwong *et al*, 1999).

Extraction of genomic DNA from whole blood

Blood samples were collected from volunteers in an EDTA-coated tube (Vacuette, Greiner Labortechnik, Austria). Lymphocytes were prepared from peripheral blood samples using a conventional method. In brief, peripheral blood samples were washed with phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄·7H₂O and 1.4 mM KH₂PO₄, pH 7.3) and red blood cells were lysed with a lysis buffer (155 mM NH₄Cl, 1 mM KHCO₃, 0.01 mM EDTA). Lymphocytes were finally collected as pellets by centrifugation. Genomic DNA was then isolated from peripheral blood lymphocytes using DNAzol[®]BD reagent (Molecular Research Center, Inc, OH, USA) following the manufacturer's instruction. Genomic DNA solution was diluted in TE buffer and its concentration was determined by UV spectrophotometry at optical density (OD) 260/280 nm. DNA was kept at 4°C and used as a positive control for PCR amplification.

RESULTS

Amplification of a short DNA fragment from the extracted genomic DNA and further test for genetic analysis using SSCP

To determine if the quality of the extracted genomic DNA was appropriate for a subsequent genetic analysis, the extracted DNA was subjected to PCR amplification of the exon 5 of the PAX8 gene, a gene coding for a transcription factor belonging to the mammalian PAX protein family (Strachan and Read, 1994), as described in Materials and Methods. Genomic DNA extracted from the whole blood of the same volunteers,

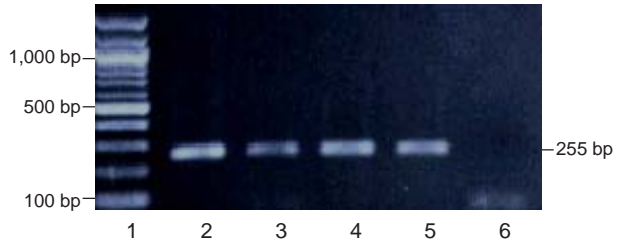


Fig 1—PCR amplification of the 255-bp fragment of the PAX8 gene. Agarose gel electrophoresis illustrated the PCR products of the PAX8 exon 5 sequence amplified from the genomic DNA extracted and purified from dried blood spots. Lane 1, 100 bp DNA ladder; Lane 2, a positive control of the PCR products amplified from genomic DNA extracted from fresh blood; Lanes 3-5, samples of the PCR analyses obtained from the genomic DNA extracted from dried blood spots; Lane 6, a negative control.

using a commercial reagent, was used as a positive control. The results illustrated that the PCR products of the expected size of 255 bp were detected in all samples (Fig 1). The quantity of the PCR products obtained from the dried blood spot extraction was roughly equal to that obtained in the positive control.

To determine if the extracted genomic DNA was appropriate for further genetic analyses, *eg* mutation determination, the PCR products then underwent SSCP analyses. Fig 2 shows a clear pattern of the exon 8 sequence compared with the PCR products amplified from the genomic DNA extracted from fresh blood.

Amplification of medium and large DNA fragments

Use of genomic DNA in genetic studies may also involve the amplification of large sequences. To verify if the genomic DNA extracted from filter paper by the developed method could be used to determine larger DNA fragments, PCR analyses were performed to amplify a 674-bp fragment of the PAX8 gene and a 1,039-bp fragment of β -globin gene. Results showed that the large DNA sequences of the expected sizes could be amplified from the DNA samples extracted by the developed method (Fig 3, A and B).

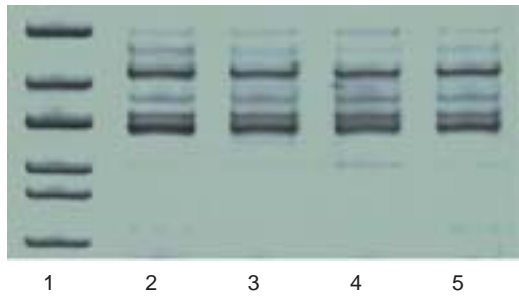


Fig 2—SSCP analyses of the PCR-amplified 255-bp fragment of the PAX8 gene. SDS PAGE gel analysis illustrated the SSCP patterns of the fragments of the PAX 8 exon 5 sequence obtained from PCR amplification of the genomic DNA extracted from blood spot specimens (lanes 3-5) compared with a positive control of the PCR products amplified from the genomic DNA extracted from fresh blood (lane 2). Lane 1 was the nondenatured 100-bp DNA marker used only to control the running period of the gel, but not to compare the size.

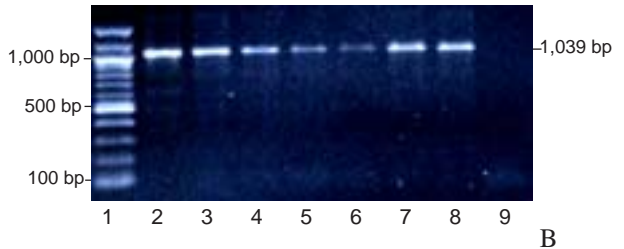
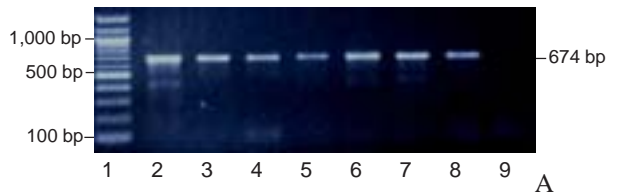


Fig 4—Amplification of genomic DNA from dried blood specimens stored under different conditions. Genomic DNA extracted from dried blood samples stored at room temperature, 4°C and -20°C were PCR-amplified to determine the 674-bp PAX8 (A) and the 1,039-bp β-globin gene (B) sequences. Lane 1, 100 bp DNA ladder; Lane 2, a positive control of the PCR products amplified from genomic DNA extracted from fresh blood; Lanes 3 and 4, PCR products obtained from samples stored at room temperature; Lanes 5 and 6, PCR products obtained from samples stored at 4°C; Lanes 7 and 8, PCR products obtained from samples stored at -20°C; Lane 9, a negative control.

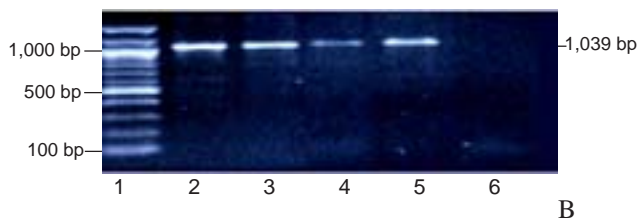
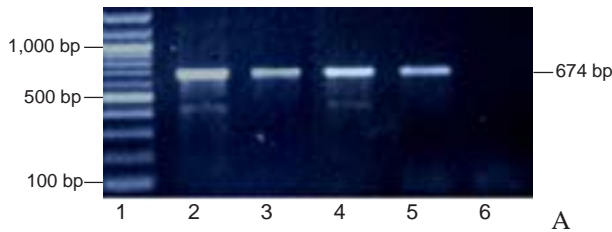


Fig 3—Agarose gel electrophoresis illustrating PCR-amplification of the 674-bp fragment of the PAX8 gene (A) and the 1,039-bp fragment of the β-globin gene (B) from the genomic DNA extracted by the developed method. Lane 1, 100 bp DNA ladder; Lane 2, a positive control of the PCR products amplified from genomic DNA extracted from fresh blood; Lanes 3-5, samples of the PCR obtained from the genomic DNA extracted from dried blood spots; Lane 6, a negative control.

Extraction of genomic DNA from dried blood spots kept under different conditions

We showed further that the developed method was appropriate for extracting genomic DNA from dried blood filter paper stored under different conditions. PCR analyses of the genomic DNA extracted from dried blood samples kept at 4°C, -20°C and room temperature were performed, using primers to amplify the 674-bp PAX8 fragment and the 1,039-bp β-globin gene. The results confirmed that the developed method was suitable for genomic DNA extraction and purification from dried blood samples stored under various conditions (Fig 4, A and B).

DISCUSSION

This report describes a simple, efficient method for extracting and purifying genomic DNA from dried blood stored on filter paper. We observed similar sensitivity and specificity of the PCR amplification from the extracted products, compared with the PCR results of the genomic DNA obtained from fresh blood. In addition, the quality of the resultant PCR products has been shown appropriate for further genetic studies, as shown by the SSCP analysis of the exon 5 PCR product of the PAX8 gene. Large DNA fragments, of at least 1 kb in size, can be determined by amplification of the DNA extracted by the developed method. Panteleeff and colleagues (1999) have also presented a method for extracting DNA from dried blood. However, their method is appropriate for determining only a very small fragment, about 140 bp, but not for the amplification of large DNA fragments, *ie* all of the attempts to amplify 0.9 and 1.2 kb DNA fragments from the extracted DNA have failed.

To our knowledge, our method is optimal and efficient for the extraction and purification of genomic DNA from dried blood specimens. DNA of a housekeeping gene, the β -globin gene, which is expected to be present in every cell, can also be detected. The ability to amplify the gene represents a likelihood that other genes of interest from the purified genomic DNA solution may be amplified. The genomic DNA obtained can also be stored for long-term use because it has been purified and all the digestive enzymes have been removed.

The method is simple and inexpensive. The benefit of the developed method would also promote the use of dried blood samples for genetic studies, especially considering collection, process and storage, which are easy and straightforward.

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