Rapid, Simple Alkaline Extraction of Human Genomic DNA from Whole Blood, Buccal Epithelial Cells, Semen and Forensic Stains for PCR

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Various protocols have been devised for the extraction of genomic DNA from human tissues and forensic stains for polymerase chain reaction (PCR) depending on such treatments as boiling, hydrolysis with proteinases, exposure to ultrasound waves, detergents or alkali (5,7,9–13,15). Alkaline extraction is among the simplest strategies. It is an efficient means for the solubilization of proteins (e.g., membrane proteins) due to the ionization of aspartic, glutamic, cysteic and tyrosine residues (4), and the primary structure of DNA is relatively stable in alkaline solution (2). Alkaline lysis at 65° or 95°C has been devised for extraction of single cells and for viral DNA in human tissues (7,13), but to our knowledge, alkaline extraction of genomic human DNA has not achieved any widespread usage. Here, we show that efficient extraction of DNA from whole blood can be performed with alkaline treatment at room temperature in a time frame as short as 1 min. Alkaline extraction of buccal epithelial cells, and blood and semen stains, can be accomplished in 5–6 min at 75°C, whereas liquid semen requires 30 min at 75°C.

Initially, the solubilizing effect of sodium hydroxide on biological material was assessed at different concentrations and temperatures using heat-denatured pellets of whole blood (5 µL of blood were dried at 90°C for 5 min). It was found that 20 µL ≥0.1 M NaOH at ≥70°C completely dissolved the pellet in 5 min, whereas water or 0.02 M NaOH had no effect even after incubation for 24 h. The extracts were neutralized with 0.02 M Tris-HCl, pH 7.5, and PCR showed that genomic DNA was liberated and accessible as a template (results not shown). Based on these results, 0.1 and 0.2 M NaOH were used as solvents throughout.

Aliquots (5 µL) of pooled whole blood from 5 individuals were incubated with 20 µL NaOH (0.1 or 0.2 M) at temperatures ranging from ambient to 90°C for 1–60 min. The reaction was stopped by the addition of 180 µL Tris-HCl, pH 7.5 (0.02 or 0.04 M, respectively) to bring the pH from 13.0 to 8.5. The quantity of the liberated DNA in the supernatant was assessed by slot-blotting and hybridization using the ACES™ 2.0$^+$ Human DNA Quantitation System (Life Technologies, Gaithersburg, MD, USA). Further, the quantity/quality of the DNA was estimated from the ability to yield PCR products and from the amount of product generated by amplification (28–35 cycles) of 414- and 1600-bp genomic segments at the human ACP1 locus (1,3,6). The 1600-bp segment (in intron 3S/exon 4) was amplified using the following primers, 5′-GGGAAAGTCTAGTTGTTAATAGCATG-3′ (sense) and 5′-GATTACTTTTTCTATTCAAATCTCTG-3′ (antisense), denaturation at 94°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 3 min. All extractions were carried out in duplicate or more. The extraction of whole blood was complete after incubation with 0.2 M NaOH for 1 min at room temperature; a longer extraction time or higher temperature did not increase the amount of liberated DNA (Figure 1A). The yield was about 65%, assuming 30 ng of nuclear DNA per microliter of whole blood (5), and the liberated DNA was of high molecular weight, as evidenced by the electrophoretic appearance (Figure 2). After a prolonged (>30 min) incubation at 75°C, the amount of PCR product that could be obtained with the extracted DNA as template decreased, indicating that the quality of the DNA was reduced [Figure 1A, lanes 4–6; and lanes 7–10 (blood stains)]. Introduction of a brief washing step (i.e., suspension of the blood in water, followed by centrifugation and before extraction with NaOH) removed 85% of the proteins in the crude blood extracts, as estimated from an elemental analysis of the content of organic oxygen, nitrogen and carbon in crude and washed extracts. Usually, 5 µL of extract were adequate in 50 µL of PCR; however, the volume of crude, unwashed extract could be increased to 50% of the PCR without significantly impairing the reaction. Extracts were stable for at least a month at 4°C as evi-

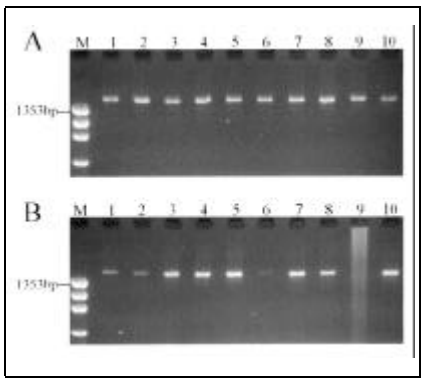

Figure 1. Extraction of human genomic DNA from whole blood, blood stains, semen, semen stains and buccal epithelial cells with 0.2 M NaOH. Agarose gel electrophoresis of 1600-bp PCR product (ACP1 locus, intron 3S) using DNA extracts as template (35 cycles): Lane assignments (from left to right): M, ϕX174 RF DNA *Hae*III-digested, 72–1353-bp size markers (Stratagene, La Jolla, CA, USA); Panel A, lanes 1–3, whole blood extracted at room temperature for 1, 5 and 20 min; Panel A, lanes 4–6, whole blood extracted at 75°C for 5, 20 and 40 min; Panel A, lanes 7–10, blood stains extracted at 75°C for 1, 5, 20 and 40 min; Panel B, lanes 1–5, semen extracted at 75°C for 10, 20, 30, 40 and 60 min; Panel B, lanes 6–8, semen stains extracted at 75°C for 1, 5 and 20 min; Panel B, lanes 9 and 10, buccal epithelial cells extracted for 6 min at room temperature and 75°C, respectively.

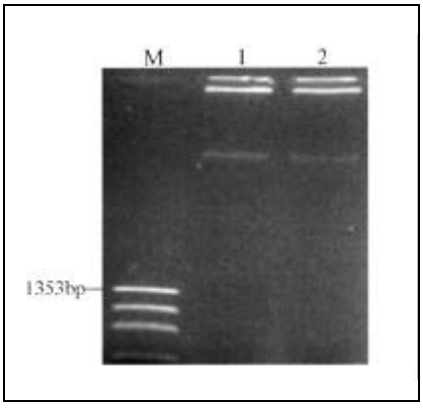

Figure 2. Stability of genomic DNA extracted from whole blood with 0.2 M NaOH. Agarose gel electrophoresis of extracts. Lane assignments (from left to right): Lane M, ϕX174 RF DNA *Hae*III-digested, 72–1353-bp size markers; Lane 1, freshly prepared extract; Lane 2, extract stored at 4°C for 1 month. Both extracts were concentrated 10× on Sephaglas™ BandPrep Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) immediately before electrophoresis (uppermost white line in lanes 1 and 2 is Sephaglas in application slot).

Table 1: Protocols for Extraction of Genomic DNA

All operations are performed in 1.5-mL microcentrifuge tubes.

Step 1. Extract the DNA

(*i*) **Whole blood.** Mix 5 μL of blood (preferably containing an anti-coagulant) with 20 μL 0.2 M NaOH and incubate at room temperature for 5 min (1 min is sufficient, but 5 min are easier to administer when several samples are processed simultaneously).

If **removal of hemoglobin and plasma proteins** from whole blood is desired, modify step $^{(i)}$ as follows: mix 5 μL of whole blood with 1 mL of distilled water and centrifuge immediately at 12 000× *g* for 1 min and discard the supernatant. Mix the dry pellet with 20 μL 0.2 M NaOH and incubate at room temperature for 5 min.

(*ii*) **Blood stains.** Mix stain (ca. 5 μL blood) with 20 μL 0.2 M NaOH and incubate at 75°C for 5 min (apply as little non-stain material as possible to ensure that the stain is completely soaked with NaOH).

(*iii*) **Semen.** Mix 1 μL of liquid semen with 20 μL 0.2 M NaOH and incubate at 75°C for 30 min and cover with oil to prevent evaporation.

(*iv*) **Semen stains.** Mix stain (ca. 1 μL semen) with 20 μL 0.2 M NaOH and incubate at 75°C for 5 min [same comment as for step (*ii*)].

(*v*) **Buccal epithelial cells.** Air-dry mouth swab at room temperature, transfer a proportion of the cotton to the tube and apply 20 μL 0.2 M NaOH (use no more cotton than can get completely soaked). Incubate at 75°C for 10 min (6 min are sufficient).

Step 2. Stop the extraction

Stop the extraction by adding 180 μL 0.04 M Tris-HCl, pH 7.5. The extract is now ready for use. Five microliters of extract are usually adequate for a 50-μL PCR.

denced by both the absence of significant quantities of degraded DNA (Figure 2) and the unchanged amplifiable property of the extract. Thus, treatment with 0.2 M NaOH seems to efficiently denature nucleases in the extract. Table 1 shows this protocol.

To further assess the applicability of the protocol for whole blood (without removal of hemoglobin and plasma proteins), extracts were also tested in PCRs involving two other human structural gene loci [GC, 186-bp fragment; ABO, 198-bp fragment (1)] and two human tandem repeat loci [D1S80, 16-bp repeat, 350–900-bp fragments; HUMTH01, 4-bp repeat, 183–221-bp fragments (8,14)]. Specific PCR products were obtained for all 4 loci for all individuals tested (up to 600).

Aliquots (1 μL) of pooled liquid semen (stored frozen at -20°C for up to 4 weeks) from 3 individuals were extracted as described above for whole blood. None or minimal DNA was liberated from the semen at room temperature, whereas incubation with 0.2 M NaOH at 75°C for 30 min or more gave reproducible results (Figure 1B, lanes 1–5). The yield was about 10% as measured by slot-blotting, but the quantity of amplifiable DNA appeared to be significantly higher as estimated from the amount of product generated by PCR. Addition of dithiothreitol (DTT) had no significant effect. Table 1 shows this procedure. The stability of the extracts was as described above for the blood.

Mouth swabs (cotton sticks) with buccal epithelial cells were obtained from five individuals. The swabs were air-dried at room temperature, an adequate proportion of the cotton (in the present case ca. 50%) was cut to allow

complete soaking with 20 µL 0.2 M NaOH and incubated either at room temperature or at 75°C. Extracts prepared at 75°C for 6 min or more gave consistent yields of amplifiable DNA, while extracts prepared at room temperature resulted in smears or no PCR product (Figure 1B and Table 1).

Blood and semen stains were prepared by spotting 5 µL of pooled blood or 1 µL of pooled semen onto clean cotton cloth. The stains were dried and stored in the dark at room temperature for 1–4 weeks. For extraction, the entirety of a stain (ca. 3 × 3 mm) was cut out and incubated with sodium hydroxide. Incubation with 0.2 M NaOH at 75°C for 5 min was adequate for the liberation of DNA for both blood and semen stains (Figure 1, A and B); in some experiments, incubation of blood stains for as little as 1 min resulted in amplifiable DNA. The stability of the extracts were as described above for blood. Blood and semen stains were prepared as described above and stored for 4 weeks. Forty stains of each were extracted using the protocol in Table 1. The extracts were tested by PCR of the 1600-bp ACP1 fragment, and in each case, a specific product was observed.

All essential experiments were repeated using potassium hydroxide instead of sodium hydroxide, and as expected, similar results were obtained. Thus, sodium hydroxide can be replaced with potassium hydroxide if desired.

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Speedo® Silicone Ear Plug "Snakes" are Effective Polyacrylamide Gel Casting Seals

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Despite the availability of sophisticated polyacrylamide gel electrophoresis (PAGE) apparatuses with casting stands and/or sealing mechanisms, some laboratories still use gel electrophoresis equipment that requires taping and binding of the casting device with clips to seal the boundaries of the gel plates (1).

Here, I describe a very inexpensive alternative to the laborious and failure-prone taping of gel casting plates. This new method offers certain advantages, including: *(i)* sealing the plates can be done rapidly and without tape; *(ii)* removal of the seal following polymerization of the gel is rapid and simple; and *(iii)* the seal is reusable and inexpensive.

The procedure for use is simple. One set of Speedo® swimmer's ear plugs (Part No. 753116-000) can be purchased for approximately $3.50 at most sporting goods stores or on the World Wide Web (WWW) (2). Four lumps of silicone putty are worked together by kneeding in the palms of the hands. Small chunks of the putty are torn from the larger mass and rolled into "snakes" on a glass plate (separate from the gel plates) until the diameter is approximately 3–4 mm and of the desired length. To seal the two sides and the bottom of the gel plates, three separate snakes are used (one large snake is cumbersome). To begin the sealing process, the plates are first assembled with spacers and secured on one side with binder clips (Figure 1). On the side opposite the clips, the seal is applied to the space between the plates and then pressed firmly, using a rolling motion of the thumb. Care should be taken not to displace the spacers. Moving from top to bottom or vice versa is recommended because some stretch of the material leads to excess at the end. A good seal is evident when a change in the appearance of the silicone/glass interface is noted upon the bonding of the silicone to the glass surface. Upon forming a seal on one side of the plates,