A FIVE MINUTE PROCEDURE FOR EXTRACTION OF GENOMIC DNA FROM WHOLE BLOOD, SEMEN AND FORENSIC STAINS FOR PCR

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INTRODUCTION

PCR does not require highly purified DNA and various methods have been devised to simplify the extraction of DNA for PCR. However, most methods consist of several steps and include harsh treatments to liberate the DNA; for example the widely used "Chelex 100® method" includes a boiling step and takes an hour or more to perform (Ohhara et al. 1994; McCusker et al. 1992; Nordvåg et al. 1992; Walsh et al. 1991).

It has been shown that incubation of isolated single cells with KOH and DTT at 65°C renders genomic DNA accessible to PCR (Li et al. 1991); also an alkaline extraction procedure for plant tissues was recently reported (Wang et al. 1993). Strong alkaline solutions exert a high denaturing and solubilizing effect on proteins due to ionization of aspartic, glutamic, cysteic and tyrosine residues (Ghélis and Yon, 1982). Incubation at alkaline pH may therefore disrupt and solubilize cell and nucleus membranes and denature nucleases whereas the primary structure of DNA is relatively stable to such treatment (Feliciello and Chinali, 1993). Furthermore, strong alkaline solutions may also dissolve denatured biological stains. For these reasons we have studied the applicability of alkaline extraction for the preparation of human genomic DNA from blood, semen and stains for PCR.

MATERIALS AND METHODS

Pooled blood drawn in sodium citrate from 5 individuals and pooled semen from 3 men with normal cell counts were used for the extraction experiments.

Extraction of DNA was performed by incubation of 5μl of blood or 1μl of semen or the equivalents of stain material with 20μl 0.1M NaOH in a 1.5ml centrifuge tube for various amounts of time and at different temperatures. The incubations were terminated by the addition of 180 μl 0.02M Tris, pH 7.5, bringing the pH of the solution from 13 to 8.5. The extracts (5μl of supernatant) were tested for the presence of liberated DNA by PCR amplification (30 cycles) of a 404 bp segment at the ACP1 locus using primers 10 and 16 as previously described (Lazaruk et al. 1993). For extracts of whole blood the quantity of liberated DNA in the supernatant was also estimated by slot-blotting and hybridisation[1]. Removal of plasma proteins and hemoglobin from whole blood was performed by mixing the blood (5μl) with 1ml of distilled water, incubation for 5 min at room temperature, centrifugation at 12,000xg for 5 min and removal of the supernatant. The pellet was extracted with 20μl NaOH as described above. This removed about 85% of the protein content as determined by elementary analysis of crude and washed extracts[2].

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[1] Kindly performed by Dr. B. Eriksen and G. Masumba using the ACES 2.0 Human DNA Quantization System (Life Technologies).

[2] Elementary analysis of Nitrogen, Carbon and Hydrogen was kindly performed by Karin Lindtog, Department of Organic Chemistry, H.C. Ørsted Instituttet, University of Copenhagen.

Blood and semen stains were prepared by spotting 5μl of blood or 1μl of semen onto clean cotton cloth. The stains were dried and stored in the dark at room temperature for 2 weeks.

RESULTS AND DISCUSSION

Initially the solubilizing power of NaOH was tested on heat denatured dry (90°C for 5 min) pellets of whole blood. It was found that incubation with 0.1 M NaOH at 70°C (or higher) for 5 min. completely dissolved the pellet whereas water or 0.02M NaOH had no effect. PCR on the neutralized extract showed that genomic DNA was rendered accessible as template. Extraction with 0.1M NaOH was hereafter attempted on whole blood and semen as well as on stains of these.

Whole blood:
Incubation of whole blood with 0.1M NaOH for 1 min or more at room temperature liberated high amounts of genomic DNA. Longer incubation time or higher temperature did not increase the yield (approx. 60%). For comparison 5μl of blood were also extracted by the "Chelex method" (Walsh et al. 1991). The yield of DNA extracted was significantly lower being about 50% of the yield of the NaOH procedure as estimated by the slot blot method (Fig. 1).

To test whether the extracted genomic DNA was generally usable for PCR, DNA segments at 3 structural loci (ACP1, GC and ABO) and two tandem repeat loci (D1S80, 16bp repeat and HUMTH01, 4bp repeat) were amplified (Lazaruk et al. 1993; Witke et al. 1993; Yamamoto and Hakomori, 1990; Thymann et al. 1993; Nellemann et al. 1994; Dissing, unpubl. results). Five μl of extract were used as template throughout. In all cases specific PCR product were obtained and the yields were consistently greater than when the same quantity of Chelex extract was used as source of DNA (Fig. 1). Silver staining of D1S80 bands as separated by polyacrylamide gel electrophoresis gave rise to some background staining. This was probably caused by the high protein content of the crude NaOH extract, and did not occur when a brief "washing step" of the blood sample was introduced prior to the NaOH extraction step.

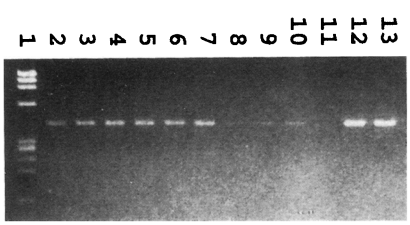
Semen:
Incubation of fresh semen with 0.1M NaOH (with or without added DTT) did not give consistently high yields of DNA at any of the temperatures tested (ambient to 90°C) as indicated by the results of PCR with the extracts. However, the yields were greatly increased if the semen was dried briefly (75°C for 5 min) prior to extraction. Incubation with NaOH was far more effective at 75°C or 90°C than at room temperature. DTT had no positive effect at elevated temperatures. The time was not critical and similar results were obtained after incubation for 5 to 60 min.

Blood and semen stains:
Incubation of the stains with NaOH at 75°C for 5 min was effective with both types of stains, whereas incubation at room temperature or prolonged incubation (> 30 min for blood stains) at 75°C or 90°C yielded less DNA or less intact DNA as indicated by a decrease in the amount of PCR product (Fig. 2).

CONCLUSION

A simple short alkaline extraction step is sufficient for extraction of genomic DNA for PCR. Incubation of 5 μl of whole blood or 1 μl of semen (or the equivalents of stain material) with 0.1 M NaOH for 5 min at either room temperature (with whole blood) or 75°C (with semen and stains) results in the release of high amounts of DNA. After the addition of 180μl

0.02M Tris pH 7.5 the extract is ready for PCR; no washing, treatment with proteases, boiling or centrifugation are required. Five μl of the 205μl extract are usually adequate as template in a 50μl PCR reaction. The extract is stable at 4°C for months indicating that endogenous nucleases are effectively denatured by the extraction process.



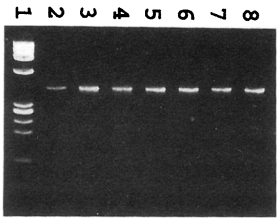


Fig. 1 (left). Comparison of DNA extracts prepared from 5μl of whole blood using different extraction conditions. Agarose gel electrophoresis of a 404bp fragment (ethidium bromide stained) PCR amplified with the human ACP1 locus as template (PCR using 5μl of DNA extract, 50μl reaction volume, 30 cycles of 94°C, 30 sec., 62°C, 30 sec., 72°C, 30 sec. DNA was extracted with 0.1M NaOH for 1 min (lanes 2,3), 5 min (lanes 4,5), 20 min (lanes 6,7) at room temperature or by the "Chelex method" (lanes 8-11). A control extract was prepared from 5μl of phenol/chloroform purified DNA (50ng/μl) by treatment with NaOH for 5 min as described above (lanes 12,13). Lane 1, ϕX 174 RF DNA HaeII digested 72-1353bp size markers (Stratagene).

Fig. 2 (right). Extracts of DNA prepared from blood and semen stains. PCR and agarose gel electrophoresis of PCR product as described in Fig. 1. Blood stains were incubated with 0.1M NaOH for 5 min. at room temperature, 75°C and 90°C (lanes 2-4, respectively). Semen stains were incubated with 0.1M NaOH at 75°C for 5, 20, 30 and 60 min. (lanes 5-8, respectively). Lane 1, size markers (see Fig. 1).

REFERENCES

Feliciello I, Chinali G (1993) A modified alkaline lysis method for the preparation of highly purified plasmid DNA from Escherichia coli. Anal Biochem 212:394-401

Ghélis C, Yon J (1982) Protein Folding. Academic Press, New York: 223-296

Lazaruk KDA, Dissing J, Sensabaugh GF (1993) Exon Structure at the Human Acp1 Locus Supports Alternative Splicing Model for f-Isozyme and s-Isozyme Generation. Biochem Biophys Res Commun 196:440-446

Li H, Cui X, Arnheim N (1991) Analysis of DNA sequence variation in single cells. Methods 2:49-59

McCusker J, Dawson MT, Noone D, Gannon F, Smith T (1992) Improved method for direct PCR amplification from whole blood. Nucleic Acids Res 20:6747

Nellemann LJ, Møller A, Morling N (1994) PCR typing of DNA fragments of the short tandem repeat (STR) system HUMTH01 in Danes and Greenland Eskimos. Forens Sci Int 68:45-51

Nordvåg B-Y, Husby G, El-Gewely MR (1992) Direct PCR of Washed Blood Cells. Biotechniques 12:490-492

Ohhara M, Kurosu Y, Esumi M (1994) Direct PCR of whole blood and hair shafts by microwave treatment. Biotechniques 17:726-728

Thymann M, Nellemann LJ, Masumba G, Irgens-Møller L, Morling N (1993) Analysis of the locus D1S80 by amplified fragment length polymorphism technique (AMP-FLP). Frequency distribution in Danes. Intra and inter laboratory reproducibility of the technique. Forens Sci Int 60:47-56

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a Medium for Simple Extraction of DNA for PCR-based Typing from Forensic Material. Biotechniques 10:506-513

Wang H, Qi M, Cutler AJ (1993) A simple method of preparing plant samples for PCR. Nucleic Acids Res 21:4153-4154

Witke WF, Gibbs PE, Zielinski R, Yang F, Bowman BH, Dugaiczyk A (1993) Complete structure of the human Gc gene: differences and similarities between members of the albumin gene family. Genomics 16:751-754

Yamamoto F-I, Hakomori S-I (1990) Sugar-nucleotide donor specificity of histo-blood group A and B transferases is based on amino acid substitutions. J Biol Chem 265:19259-19262