Int J Legal Med (1995) 108:154–156

TECHNICAL NOTE

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Extraction of high quality DNA from bloodstains using diatoms

Received: 6 June 1995 / Accepted: 11 August 1995

Abstract A simple method is described for the extraction of high quality DNA for PCR amplification. The DNA was extracted by using Chelex-100 ion exchange resin or a special cell lysis buffer containing proteinase K. For further purification the DNA was bound to silica in the presence of a chaotrophic agent. Hence it is possible to unlimitedly wash the bound DNA and inhibitory substances are removed. By using diatoms as a source of silicates, this method is very economical and can therefore be used as a routine method.

Key words DNA extraction · Diatoms · PCR · Stain analysis

Introduction

PCR amplification is increasingly gaining importance in forensic science. Despite the rapid development of highly informative PCR systems, especially of the STRs [1,10], the main problem in forensic stain analysis is not the amplification but the DNA extraction. Although sufficient amounts of DNA can be obtained with the methods of extraction available, the extracted DNA is often not amplifiable by PCR [6]. This problem can be attributed to DNA degradation products with less than 100 bp and to PCR inhibitory agents that are copurified with the high molecular DNA. It was therefore our aim to develop a DNA extraction method for stains which eliminates these inhibiting factors. For this purpose we combined the advantages of the simple Chelex-100 extraction method with the exellent DNA binding capacity of silica [3,4].

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Materials and methods

Fresh bloodstains of FES type 11/12 were applied to 16 different materials: paper, timber, tree-bark, nylon, white soft tissue paper, green soft tissue paper, white cotton, coloured cotton, black denim, green cotton, imitation leather, blue wool and black silk. All these materials were new and were not washed prior to preparation of the bloodstains. Additionally a smoked cigarette and a hair from the same person were included in the investigation.

The extraction of the DNA was carried out with a modified Chelex-100 extraction method [4, 9] or by digestion with proteinase K in a lysis buffer.

For the Chelex-100 extraction a 1-mm^2 piece of the bloodstain was used. Bloodstains of the same size were incubated in 200 µl of lysis buffer (0.1 M NaCl, 0.02 M Tris-HCl, 0.05 M EDTA, pH 8.0, 2.5% SDS) and 0.5 mg/ml proteinase K at 37°C for 1 h.

For further purification of the DNA, 50 µl of the Chelex-100 extract or of the lysis buffer/proteinase K extract was incubated with 900 µl L6-buffer (40 mM Tris-HCl, pH 6.4, 17.5 mM EDTA, pH 8.0, 1% Triton X-100, 40 mM guanidine(thiocyanate) and 50 µl 20% diatoms in 1% HCl (Sigma) for 5 min [3]. Subsequently the extract was gently pushed into a mini-column (Promega) with a 2 ml syringe, washed the same way with 2 ml wash buffer (2.5 mM EDTA, pH 8.0, 5 mM Tris-HCl, pH 7.5, 100 mM NaCl, 60% ethanol) and finally washed with 2 ml 80% ethanol. The mini-column was then transferred to a 1.5 ml microcentrifuge tube and centrifuged for 20 s at 1200 × g to remove the ethanol. To dry the diatoms completely the mini-column was incubated at room temperature for 10 min. For elution of the DNA the mini-column was transferred in a new micro centrifuge tube and 50 µl sterile water was applied. After 5 min of incubation the DNA was recovered from the diatoms by centrifugation and stored at 4°C. From the DNA extracted using Chelex-100, Chelex-100/diatoms or proteinase K/diatoms 2 µl were amplified using the FES-system as described previously [7] in a Perkin Elmer 480 DNA-Thermal-Cycler. Gel electrophoresis, silver staining and typing was carried out as described previously [2,7]. The extracted DNA was quantified by slot blot analysis as described in the protocol from Gibco-BRL. An aliquot of 15 µl was applied to 0.9% agarose (FMC) gel electrophoresis and the DNA was visualized by ethidium bromide staining.

Results

The results of analysis of DNA recovered from the 16 substrates demonstrated that in all cases sufficient DNA for PCR amplification could be extracted from the 1 mm^2 piece of the sample. The average DNA yield from all sub-

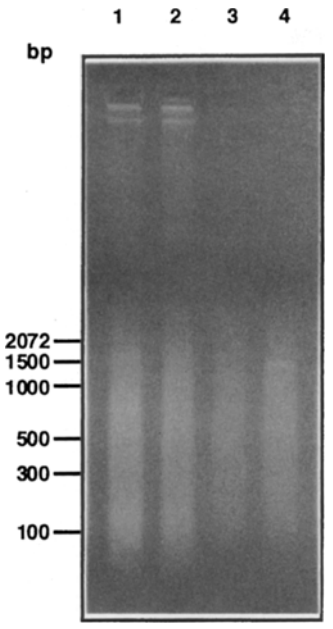

Fig. 1 DNA extracted from 2 bloodstains with the proposed methods. *Lanes 1* and *2*, DNA extracted with Chelex-100; *lanes 3* and *4*, DNA from the two Chelex-100 extracts additionally extracted using diatoms. Electrophoresis has been performed at 80 V for 60 min in 0.9% agarose gel in TBE

strates obtained by the extraction using Chelex-100 was approximately 250 ng, by the extraction using Proteinase K-diatoms 100 ng and by the extraction using Chelex-100 and diatoms only 15 ng.

The analysis of the extracted DNA on an agarose gel showed that low molecular DNA (< 60 bp) and high molecular DNA was lost during purification using diatoms (Fig. 1).

As a control of amplification the PCR products were typed by a native, high-resolution polyacrylamide gel electrophoresis. All successfully amplified samples could be classified correctly in the system FES. Only 42% of the samples extracted using Chelex-100 were amplified by PCR without problems, whereas a PCR amplification could be achieved in 82% of the samples extracted using Proteinase K/diatoms or Chelex-100/diatoms (Table 1). The 2 substrates tree-bark and black denim turned out to be the most troublesome stains. From these substrates we were not able to gain amplifiable DNA using Chelex-100 alone. In contrast, DNA from 12 of 14 samples from tree-bark and 10 out of 14 samples from black denim extracted using proteinase K/diatoms was amplified without complications. When the DNA was extracted using Chelex 100/diatoms, we were able to amplify DNA from 13 out of 14 samples from both substrates by PCR. For other STRs (e.g. VWA, TH01, F13B and CD4) and AMPFLPs (D1S80 and YNZ22) we achieved similar results in routine use (data not shown).

To determine the influence of the amount of extract on the PCR, different quantities of Chelex-100 extracts and extracts using Chelex-100 and diatoms were used for PCR. The success of PCR amplification of DNA extracted with the Chelex-100 method was strongly dependent on the amount of extract used. The largest proportion of successful amplifications was achieved by using 2 µl of the extract for PCR. Using more or less Chelex-100 ex-

Table 1 STR FES/FPS amplification of DNA derived from different sources and extraction methods (+ good amplification, + – amplification sometimes not possible, – no amplification possible; for amplification and electrophoresis see "Materials and methods")

Source of DNA	Chelex-100 extraction	Lysisbuffer-proteinase-K-extraction and diatoms	Chelex 100 extraction and diatoms
Cigarette	+ –	+	+
Paper	+	+	+
Timber	+ –	+	+
Hair	+	+	+
Tree bark	–	+ –	+ –
Nylon	+	+	+
Green soft tissue paper	+	+	+
Soft tissue paper (white)	+ –	+	+
Cotton (white)	+	+	+
Cotton (coloured)	+ –	+	+ –
Black denim	–	+ –	+ –
Cotton (green)	+	+	+
Cotton (black)	+ –	+	+
Imitation leather	–	+	+
Wool (blue)	+	+ –	+
Silk (black)	+	+	+

Table 2 Influence of extract volumes (Chelex-100 extract vs Chelex-100/diatoms extract from different sources) on PCR amplification, (+ good amplification, – no amplification possible; for amplification and electrophoresis see "Materials and methods")

Source of DNA	Chelex			Chelexdiatoms		
	1 µl	2 µl	5 µl	1 µl	2 µl	5 µl
Cigarette	–	–	–	+	+	+
Paper	–	+	+	+	+	+
Timber	–	+	–	+	+	+
Hair	–	+	+	+	+	+
Tree bark	–	–	–	+	+	–
Nylon	–	+	–	+	+	+
Green soft tissue paper	–	+	–	+	+	+
Soft tissue paper (white)	–	+	–	+	+	+
Cotton (white)	–	+	–	+	+	+
Cotton (coloured)	–	–	–	+	+	+
Black denim	–	–	–	+	+	+
Cotton (green)	–	+	–	+	+	+
Cotton (black)	–	–	–	+	+	+
Imitation leather	–	–	–	+	+	+
Wool (blue)	–	+	–	+	+	+
Silk (black)	–	+	–	+	+	+

tract for PCR the proportion of successful amplifications greatly decreased. In contrast the amount of DNA extracted using diatoms exerted much less influence. Only the troublesome sample from tree-bark could not be amplified with 5 µl of the extract (Table 2).

Discussion

Despite the greater loss of DNA during the extraction using diatoms, due to the lower binding properties of the diatoms for nucleotides and oligonucleotides [3], the results of this study showed the clear advantages of the additional purification of the Chelex-100 extract by diatoms.

By means of the additional purification with diatoms, which leads to a removal of most inhibitory substances and short DNA fragments that inhibit especially the first cycles of PCR [8], we were able to increase the proportion of reproducible amplifications by PCR almost twofold compared to the conventional Chelex-100 extraction. The stains used for the DNA extraction measured only 1 mm^2 and were therefore much smaller then those required for a reproducible amplification using other extraction protocols [5].

The better reproducibility of the amplification after the purification step using diatoms turned out to be due – among other things – to the wider range in the amount of extract used. The independence from the volume of extract compared to the Chelex extraction without further purification can also be attributed to the lower concentration of PCR inhibitors.

The fact that neither proteinase K/lysis buffer extraction nor extraction using diatoms without prior Chelex-100 extraction yielded amplifiable DNA shows that essentially the additional extraction of the DNA with diatoms leads to the high rate of amplification by PCR. The best reproducible amplification was obtained using the combination of Chelex-100 with diatoms; this was made especially obvious by the amplification of extracted DNA from tree-bark and black denim. Although it leads to the lowest DNA yields, this combination offers the best reproducible PCR amplification. It combines the degradation inhibiting properties of Chelex-100 [9] with the additional washing possible using diatoms. We could show that this combination is a quick, inexpensive and reproducible alternative method for DNA extraction. Especially for difficult stains when the Chelex-100 extraction alone fails, it is possible to use diatoms for additional purification. In addition, it is possible to enhance the efficiency of the PCR by combining the extraction of DNA using diatoms and guanidine thiocyanate with many other extraction protocols, e.g. that based on phenol chloroform.

Acknowledgements We thank C. Diem and I. Kirleis for their comments on the manuscript, and C. Cuno, G. Hinkelmann and C. Laudenbacher for technical assistance.

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