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Applications of thiopropyl sepharose 6B for removal of PCR inhibitors from DNA extracts from different sources

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Abstract

This study presents applications of thiopropyl sepharose 6B (TS) for removal of PCR inhibitors from DNA extracts from a body recovered from the sea and other sources encountered in forensic casework.

Keywords: Removal of PCR inhibitors; Thiopropyl sepharose 6B; Forensic science

1. Introduction

Attempts to amplify DNA from forensic samples by PCR are often hampered by the presence of inhibitors of *Taq*-polymerase. A possible strategy to remove such inhibitors is based on the use of the affinity resin thiopropyl sepharose 6B (TS), which can undergo exchange with disulfide dyes that often are used by the clothing industry to dye cotton fabrics [1]. TS has previously been reported to be effective in the removal of inhibitors in DNA extracts originating from stains recovered from clothing [1,2]. In the current study, we investigated the use of TS for the removal of PCR inhibitors from DNA extracted from a number of different sources including a thigh bone from a body recovered from the sea after 4 years in seawater. In addition, we present an investigation on the loss of DNA associated with TS purification.

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2. Materials and methods

An extensively decomposed body part was recovered in December 2000 by a trawler north of the Danish island Sejerø. Based on the clothing, it was anticipated that the remains were those of a 49-year-old man who was missing in the area after a fishing expedition in late 1996. To confirm this, DNA was extracted from the thigh bone using proteinase K and phenol/chloroform extraction followed by concentration on Centricon 100 filters. For comparison, DNA was also extracted in the same manner, from paraffin-embedded tissue originating from the suspected victim. The amount of human DNA extracted was quantified by slot-blot analysis followed by hybridisation with an alkaline phosphate-conjugated D17Z1 probe. The DNA extract from the thighbone was treated with TS. Briefly, TS beads were agitated and hydrated in milli-Q water. Then, the sample to be purified was agitated with the hydrated TS beads at room temperature for 15 min, followed by centrifugation and recovery of the supernatant. The TS beads were then washed in TNE buffer and the supernatant was recovered after centrifugation. Finally, the DNA from the combined supernatants was concentrated on Centricon 100 filters. The DNA extracts were amplified with the AmpF*l*STR SGM Plus kit (Applied Biosystems). In a similar way, TS purification was used on a total of 12 different samples from eight different cases submitted to our laboratory by the Danish police. In all cases, TS purification was used only when amplification attempts had failed and quantification had shown that sufficient DNA was present. In addition to the TS purifications performed on samples suspected to contain PCR inhibitors, a study on the loss associated with TS purification was performed. For this study, a total of 19 samples containing either high or low quantities of DNA were selected. All the samples chosen for this study were quantified and successfully amplified with the SGM Plus kit before and after the TS purification.

3. Results and discussion

The quantification of the DNA extracts from the thighbone revealed that the extraction procedure had resulted in sufficient DNA (2.7 ng) for a subsequent PCR analysis. Nevertheless, attempts to amplify DNA from the extract from the thighbone was fruitless, while amplification of the extracts from the paraffin embedded tissue yielded a full profile with the SGM Plus kit. After purification with thiopropyl sepharose 6B, amplification of the DNA extract from the thighbone was successful. The DNA-profiles obtained from the paraffin embedded tissue of the 49-year-old man and the thighbone matched each other and the results could be reported. A significantly enhanced DNA profile was also obtained from seven of the additional 11 extracts purified with TS. The extracts successfully purified with TS originated from a broad range of sources, including saliva on an envelope and various types of biological stains deposited on different types of clothing. With the remaining five extracts suspected to contain inhibitors, the TS purification resulted in insufficient amounts of DNA for subsequent PCR analysis. In the study investigating the loss of DNA, all samples yielded a full SGM Plus profile both before and after TS purification. Thus, no contamination was associated with TS purification. The quantification revealed that substantial amounts of DNA were lost during the TS purification

Table 1
Loss of DNA associated with TS purification

	Mean total ng DNA (±S.D.)	
	Low quantity DNA (n=10)	High quantity DNA (n=9)
Before TS purification	6.0 (±2.1)	499.3 (±307.2)
After TS purification	0.9 (±0.7)	56.6 (±44.8)

(Table 1). We conclude that TS purification was effective to remove PCR inhibitors from DNA extracted from a broad range of sources including that of a human bone in an extended state of decomposition after 4 years in seawater. However, TS purification should be used only on extracts with an abundance of DNA, since the method is associated with a substantial loss of DNA.

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