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SHORT COMMUNICATION

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Application of thiopropyl sepharose 6B for removal of PCR inhibitors from DNA extracts of a thigh bone recovered from the sea

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Abstract PCR amplification of DNA from forensic samples often proves difficult due to the presence of inhibitors of the polymerase chain reaction. One possible way to remove PCR inhibitors from a DNA extract is the use of the affinity resin thiopropyl sepharose 6B (TS), which has been used previously for the removal of PCR inhibitors in DNA extracts originating from stains on clothing. Here we show that TS is efficient also for the removal of inhibitors from PCR extracts from a highly decomposed human thigh bone. TS treatment, however, leads to a substantial loss of DNA making the technique best suited when substantial amounts of DNA are present.

Keywords Removal of PCR inhibitors · Thiopropyl sepharose 6B · Forensic science

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 PCR inhibitors in DNA extracts 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 thigh bone from a body recovered from the sea after 4 years in seawater. Also, TS purification was used on DNA extracts from a number of different sources. In addition, we present an investigation on the loss of DNA associated with TS purification.

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Materials and methods

An extensively decomposed body part was recovered by a trawler north of the Danish island Sejerø in December 2000. Based on the clothing, it was assumed that the remains were those of a 49-year-old man who was lost in the area during a fishing expedition in late 1996. To confirm this, DNA was extracted from the thigh bone using proteinase K and phenol/chloroform extraction [3] followed by concentration on Centricon 100 filters (Millipore) according to the manufacturers instructions. 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 slotblot analysis followed by hybridisation with an alkaline phosphate-conjugated D17Z1 probe [4]. The DNA extract from the thigh bone was treated with TS [1, 2]. An equivalent of 100 µl dry volume of TS beads (Thiopropyl sepharose, Sigma cat. no. T8387) was hydrated by addition of 1 ml of sterile water followed by agitation at room temperature for 5 min. Then, the beads were centrifuged for 2 min. at 13,000 rpm, and the supernatant was removed. This washing step was repeated a total of 3 times and the final wash supernatant was discarded. The hydrated beads were then resuspended in 200 µl of sterile water, 200 µl of this suspension was transferred to a new tube and centrifuged for 2 min at 13,000 rpm followed by removal of the supernatant. This procedure yielded approx. 100 µl hydrated bead preparation. After preparation of the TS beads, the volume of the sample to be purified was adjusted with sterile water to 400 µl and combined with the hydrated beads. The combined beads and sample were gently agitated for 15 min at room temperature and centrifuged for 5 min at 13,000 rpm. The supernatant was transferred to a 1.5 ml microcentrifuge tube. The TS beads were washed in 100 µl TNE buffer (10 mM Tris, 1 mM EDTA, 100 mM NaCl) and the supernatant was recovered after centrifugation for 5 min at 13,000 rpm. Finally, the DNA from the supernatants was pooled and concentrated on Centricon 100 filters to a final volume of 60 µl.

The DNA extract was amplified with the AmpF*l*STR SGM Plus kit (Applied Biosystems) according to the manufacturers instructions. In a similar way, TS purification was used on a total of 12 different samples from 8 different crime cases. In all cases, TS purification was used only when amplification attempts had failed but quantification had shown that sufficient DNA (0.5 ng) was present. All TS purifications included a negative control in which sterile water was used instead of a DNA extract.

A study on the loss associated with TS purification was performed on 37 samples containing either high or low quantities of DNA. Since TS is not guaranteed to be DNA-free, this study also served as a confirmation that the TS treatment did not introduce any contamination. Prior to TS purification, all samples had been amplified with the AmpF/STR SGM Plus kit. One subset of samples (n=19) was treated with TS purification as described above, while on another subset (n=18), the final concentration of DNA following the TS purification was performed with Microcon YM-100 filters (Millipore) instead of Centricon 100 filters. All the samples chosen for this study were quantified by slotblot as described above before and after the TS purification. The samples containing sufficient DNA (0.5 ng) after TS purification were then retyped with the AmpF/STR SGM Plus kit. The ratio between the amount of DNA in each sample after and before purification was calculated by dividing the total amount of DNA after purification by the total amount of DNA before purification. The Mann-Whitney U-test was used to test the significance of the differences between ratios from samples concentrated with Centricon and Microcon filters. P values <0.05 were considered significant.

Results and discussion

The quantification of the DNA extracts from the thigh bone 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 thigh bone were fruitless, while amplification of the extracts from the paraffin-embedded tissue yielded a full profile with the AmpF/STR SGM Plus kit. After purification with thiopropyl sepharose 6B, amplification of the DNA extract from the thigh bone was successful. The DNA profiles obtained from the paraffin-embedded tissue of the 49-year-old man and the thigh bone matched each other (Fig 1 shows the Genotyper plots obtained from the STR analysis of the thigh bone and the paraffin-embedded tissue).

DNA from 12 different sources was also TS treated. After TS treatment, 5 extracts contained too little DNA for further investigation. Clear DNA profiles were obtained with AmpF/STR SGM Plus amplification on the remaining seven TS treated DNA extracts that originated from biological stains on dyed clothing and one case of saliva on an envelope.

In the study investigating the loss of DNA, all samples subjected to AmpF/STR SGM Plus typing before and after TS purification (when sufficient amounts of DNA were present after TS) yielded a full profile before TS purification. The quantification revealed that substantial amounts of DNA were lost during the TS purification (Table 1). This finding is in accordance with a previous study [5], which reported an extensive loss of DNA associated with the use of Centricon 100 filters. This was especially the case when Centricon 100 filters were used, while Microcon filters gave a better yield of DNA. In the TS purification procedure, the DNA extracts were dissolved in a total volume of approx. 500 µl prior to the concentration step with Centricon or Microcon. This relatively dilute suspension of DNA was not suitable for quantification by slotblot. Thus, it was not possible to determine whether the majority of the DNA was lost along with the TS beads or in the concentration step with Centricon or Microcon.

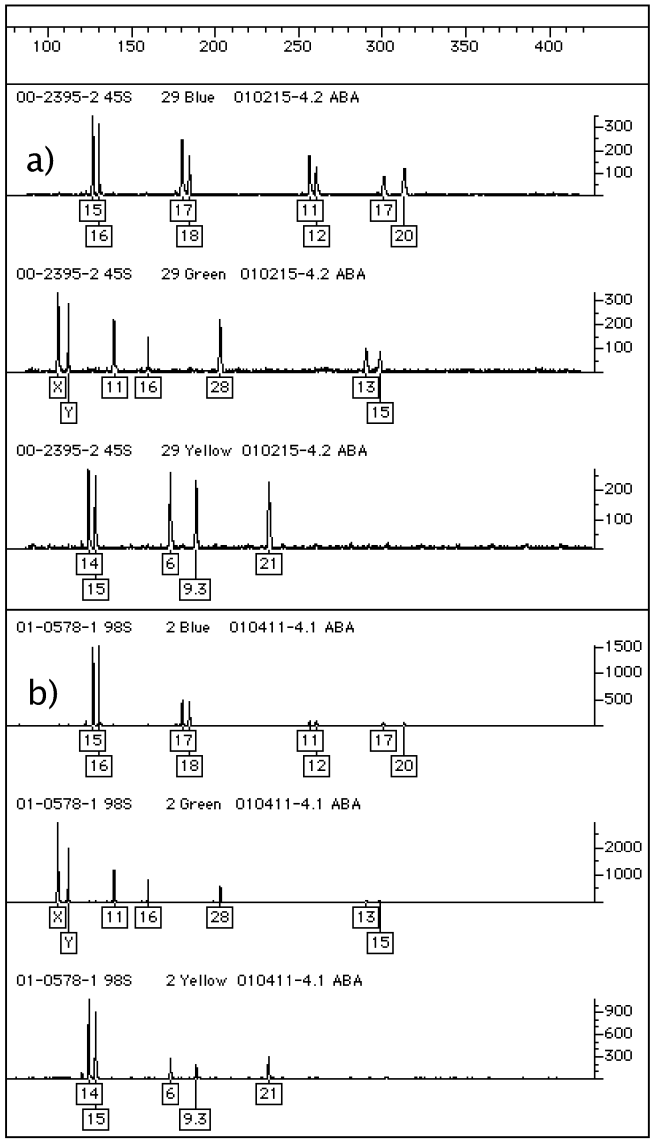

Fig. 1 Results of amplification with the AmpF/STR SGM Plus kit of DNA extracts from the thigh bone (*panel a*) and the paraffin-embedded tissue (*panel b*)

No contamination was associated with TS purification in our study. However, since TS is not guaranteed to be DNA-free, it should be validated that each batch of TS does not result in contamination. In addition, negative controls should be included with each separate TS purification procedure.

An alternative approach to the use of TS treatment could be to include the use of Qiagen spin column when extracting DNA from putrefied bones. Such an approach was reported by Crainic et al. [6] who used a modified Qiagen procedure to extract DNA from bones recovered after 3 years in freshwater followed by successful STR typing. The TS purification procedure differs from the Qiagen extraction procedure in that it is intended for use on already existing DNA extracts obtained by for example by Chelex extraction [7] or organic extraction. Thus, the TS

Table 1 Average DNA content in the samples before and after TS purification. In addition, the ratios between DNA content after and before TS purification were calculated

	Mean total ng DNA (±SD)			
	Concentrated with Centricon		Concentrated with Microcon	
	Low quantity DNA (n=10)[1]	High quantity DNA (n=9)	Low quantity DNA (n=9)	High quantity DNA (n=9)
Before TS purification	6.0 (±2.1)	499.3 (±307.2)	5.1 (±2.7)	504.7 (±215.1)
After TS purification	0.9 (±0.7)	56.6 (±44.8)	1.7 (±1.9)	205.1 (±125.6)
Ratio[2]	0.18 (±0.18)[a]	0.15 (±0.13)[b]	0.27(±0.26)[c]	0.42 (±0.23)[d]

[1] n number of samples.
[2] Ratio: ng DNA after purification/ng DNA before purification.
[b] vs. [d] $P<0.01$.
[a] vs. [c] not significant.

purification procedure is more flexible in the sense that it can be applied more broadly to DNA extracts obtained from forensic samples not beforehand expected to contain PCR inhibitors.

We conclude that TS purification was effective in removing PCR inhibitors from DNA extracted from a broad range of sources including that of a human bone in an advanced state of decomposition after 4 years in seawater. However, TS purification is best suited for extracts with an abundance of DNA because the method is associated with a substantial loss of DNA. As an alternative, the use of TS purification on DNA extracts with relatively low amounts of DNA could be combined with attempts to improve the specificity and effectivity of the PCR reaction [8], or the analysis of short tandem repeats on the Y-chromosome [9].

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