DNA-Profiling on carpeting - Methods of purification, restriction and detection

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Introduction

In practice, bloodstains on carpeting are not very often submitted for investigation (approx. 5 % of all stained materials). However, carpeting is frequently very soiled or has been treated chemically (cleaned). In contrast to bloodstaines on smooth surfaces or cotton, DNA-extraction and restriction from bloodstaines on soiled and highly absorbent materials is often difficult (Holtz and Olek, 1987; Kantner et al, 1988; Prinz and Berghaus, 1990; Scheithauer and Weisser, 1991a). Problems of identification of DNA from bloodstained materials previously treated with purification media have been demonstrated by Scheithauer (1991b).

The aim of the present study was to test and, if possible, improve the extraction and purification procedures applied to bloodstaines on carpeting. A further objective was to determine the degree, on which extraction depends upon the substrate material and its composition.

Material and methods

A total of 160 extractions was performed on bloodstains applied to five different makes of carpeting (each make available factory-new, soiled, cleaned or stored under humid conditions). The size of the stain was that produced by a drop of blood. The bloodstains were first incubated in extraction buffer (10.0 mM Tris, 10.0 mM EDTA, 100.0 mM NaCl, pH 8.0) for 3 hours at room temperature then treated with Proteinase K (final concentration 100 μg/ml) for 1 h at 50 C and 4 h at 37 C. Genomic DNA was isolated by Phenol/Chloroform extractions or by deproteinisation with 6M NaCl solution. DNA precipitation was carried out with ethanol under standard conditions. Further purification occured by membrane dialysis against TN-buffer (10.0 mM Tris, 10.0 mM NaCl, pH 8.5). The amount of DNA extracted was estimated by comparing with a concentration standard in agarose gel. Finally, the DNA was restricted with *Hinf* I (10 U/μg DNA) in a volume of 40 μl containing 0.25 % BSA (Pflug et al, 1991). In some of the extracts, RFLP analyses using two single-locus systems (MS43a and YNH24) and investigations with the AmpFLP system D1S80 were performed.

Results

The results of the examinations showed that the improved procedure yielded an average of 10 % more DNA than the standard protocol. The amount of DNA extracted from the bloodstains on the carpeting was highly dependent on the material of the carpeting (Fig. 1a). The difference between carpeting 3 (wool) and carpeting 5 (short and close polyamide fibre pile), for example, was highly significant. In general, the highest DNA yield was achieved from new, untreated carpeting, and the lowest yield from carpeting stored under humid conditions. The results obtained are shown in Fig. 1b. Fig. 2 and Fig. 3 show the results of hybridisation examinations using the probe MS43a. Fig. 4 shows the results of an experiment using the AmpFLP system D1S80.

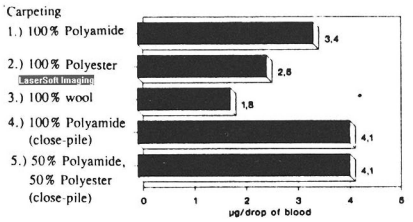

Fig. 1a: Comparison of amounts of DNA extracted from bloodstains on different carpeting makes

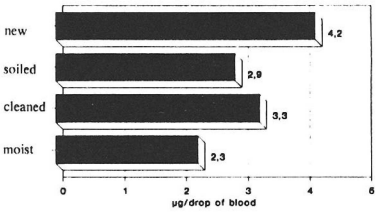

Fig. 1b: Comparison of amounts of DNA extracted from bloodstains on differently treated carpetings

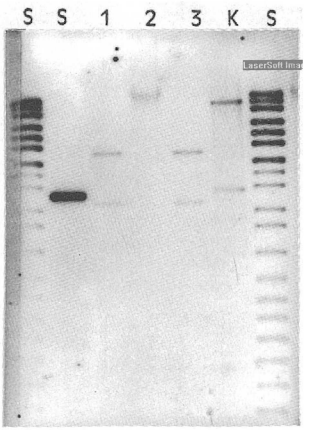

Fig. 2: Hybridisation of DNA extracted from bloodstains on differently treated carpeting
1: DNA from bloodstain on new carpeting
2: DNA from bloodstain on soiled carpeting
3: DNA from bloodstain on cleaned carpeting
S=molecular weight standards
K: K562-DNA restricted with *Hinf* I

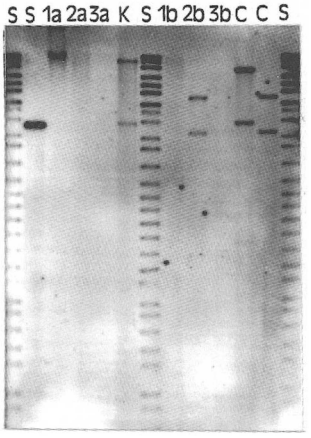

Fig. 3: Hybridisation of DNA from one donor extracted from bloodstains on three different carpeting makes
1a, 2a, 3a: restricted without BSA (restriction inhibited)
1b, 2b, 3b: restricted with 0.25% BSA (complete restriction in lane 2b)
S: molecular weight standards
K: K562-DNA restricted with *Hinf* I
C: control-DNA from two donors (bloodstain on cotton)

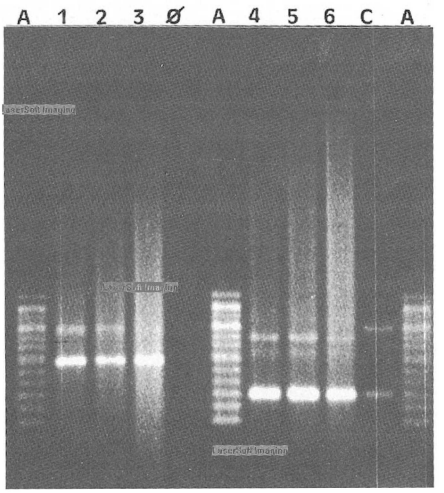

Fig. 4: Amplification of D1S80 locus
1-6: DNA from bloodstain on three carpeting makes (2 persons)
A: Allelic Ladder
C: control-DNA

Discussion

Although the extraction procedure described above produced a better yield of DNA, the number of complete restrictions remained about equal. As was expected, the largest amount of DNA was extractable from new carpeting, the least amount from carpeting stored under humid conditions.
The influence of the stained material was considerably greater than expected. Clearly less DNA can be extracted from absorbent materials (e.g. wool, carpeting sample 1) than from close-pile, less absorbent carpeting (carpeting samples 4 and 5).
In the case of partially degraded DNA, good results were obtainable in some cases using the two single-locus probes, but using the AmpFLP system produced considerably better results.
The present study has again confirmed the known fact, that BSA greatly improves DNA-restriction.

References

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