THREE VNTR AND Y-CHROMOSOME IDENTIFICATION FROM BIOLOGICAL STAINS

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INTRODUCTION

The development of the PCR for amplifiyng DNA sequences has raised the possibility that variable number of tandem repeats (VNTR) and other DNA polymorphisms might be analyzed even in samples where either the sensitivity, the quantity, or the level of degradation would, have represented an unbrechable obstacle with other methods.

In this paper, we propose a rapid and efficent method for DNA extraction from limited amounts of human biological stains of blood, semen and saliva.

The purpose of the work was to identify a simple DNA extraction procedure suitable for subsequent PCR-based DNA profiling that could be applied to the different biological stains mentioned above.

With this procedure we were able to amplify MCT 118, Apo B, YNZ 22 and Y-Chromosome.

MATERIALS AND METHODS

Sample preparation

We considered blood and semen prepared from 5 to 100 µl of whole blood and seminal fluid of known genotype, respectively. The stains had been stored in a paper bag at room temperature prior to analysis.

The saliva stains were principally taken from cigarette butts (smoked by individuals with known genotype) and stored at room temperature in a paper bag up to 1 month prior to analysis.

Incubation

Bloodstains: a pair of stains (A and B) of equivalent age and size were first cut into small pieces and introduced into an eppendorf tube and then incubated as follows:

stain A with 1 ml 0.85% NaCl for 3 h at room temperature;

stain B with 1 µl 0.85% Nacl + 100 µl buffer K (25 mM MgCl2, 20mM Tris pH8.5, 0.5% Tween 20) + 10 µl Proteinase K (10 mg/ml) for 3 h in a water bath at 65°C.

Semen stains: the same preliminary procedure for bloodstains was adopted. The stains (A and B) were subsequently incubated overnight in a water bath at 65°C as follows:

stain A with 1 ml 0.85% NaCl + 0.5% SDS + 40mM DTT+ 10 µl Proteinase K +100 µl buffer K;

stain B with 1 ml lisys buffer (10 mM Tris-HCl pH 8, 10mM EDTA, 100mM NaCl).

Cigarette butts: the buccal end of the paper covering and filter were removed, cut into small pieces, and introduced into an eppendorf tube. Incubation was in a water bath for 3 h after the addition of:

1 ml 0.85% NaCl+ 100 µl buffer K + 10 µl Proteinase K.

DNA Extraction

Following the incubation, the same procedure was used for all the biological stains under investigation. The supernatant was removed and dispensed into another eppendorf tube, while the pieces of stains were rinsed with 500 µl of 0.85% NaCl before being added to the eppendorf containing the supernatant. The solution was centrifuged at 13000 rpm for 15 min., the supernatant discarded, and the eppendorf inverted to allow the pellet to dry. Between 40 and 70 µl of dH_2O were subsequently added to the eppendorf tube: the exact amount depending on the size of the original stain employed (see results).

The contents of the eppendorf tube were then boiled for 10 min. and the DNA amplified using PCR.

Amplification and typing

PCR amplification of the DNA was performed using previously published conditions and primers. The following systems were considered: MCT 118 (1), Apo B (2), YNZ 22 (3) and Y-Chromosome (4). In order to amplify each extraction, 30 µl of the final suspension were used in a total volume of 50 µl of PCR mix.

PCR products were separated by electrophoresis in 1x TBE Buffer using 6% polyacrylamide (29:1 Acrylamide/Bis) and for Apo B, 8% for MCT 118, YNZ 22 and Y-Chr. Electrophoresis was performed at 150 V in a C.B.S apparatus for about 2 h (depending on the genetic system). The separation distance was 10 cm. The marker used was Marker VI Boehringer.For each amplified sample the amount loaded was 20 µl.

The amplification products were visualized directly after staining the gel in ethidium bromide.

As the gels were difficult to read, the silver staining procedure described by Budowle et al.(1) was adopted.

RESULTS AND DISCUSSION

Bloodstains: it was observed that DNA could be extracted from 1 to 2 day-old bloodstains made from at least 40 µl of blood by incubating the stain with 0.85% NaCl for 3 h at room temperature. However, effective DNA extraction with the smaller or older stains considered could only be achieved with the addition of Proteinase K and incubation at 65°C for 3 h.

The amount of distilled water to be added to the extracted DNA depended on the size of the bloodstains used: with stains containing up to 40 µl of blood 40 µl of dH_2O were enough, larger stains required up to 70 µl. The latter amount is sufficient for two amplifications (Fig.1).

Semen stains : with both buffers it was possible to extract DNA even from very small stains of semen (5-10 µl); furthermore, with the larger stains the incubation period could be safely shortened to 6 h.

The observations made regarding the quantity of dH_2O to be added to the DNA extracted from stains of blood are also applicable to DNA extracted from semen stains.

The above procedure was also found to be effective with mixtures of seminal and vaginal fluid, enabling differential extraction of seminal and vaginal DNA from a vaginal swab when amplifying AMP-FLP systems (Fig.2).

Saliva : it was found that good results could be achieved by incubation with 0.85% NaCl and Proteinase K, at 65°C for 3 h. The volume of distilled water added in the last step of the procedure was 40 µl (Fig.3).

Our results indicate that DNA can be extracted from the blood and saliva stains considered using 0.85% NaCl and Proteinase K by changing only the incubation time and temperature according to the nature and the age of the stain. However, semen stains required, as previous Authors have demonstrated (5), the addition of SDS and DTT to the incubation step.

Furthermore the possibility of using silver staining validating the reliability of these PCR markers for forensic application.

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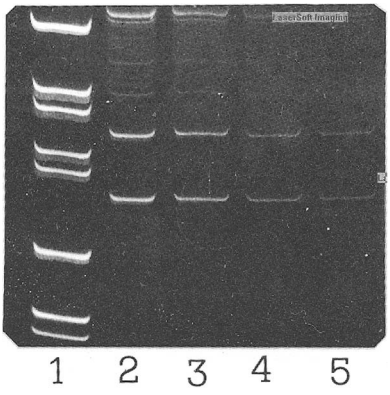

Fig. 1. Amplification of DNA extracted from bloodstains. SystemYNZ 22. Lane 1: Marker VI (Boehringer); lane 2: control DNA extracted from blood; lanes 3, 4 and 5 : extraction from 75 µl, 25 µl and 10 µl bloodstains respectively.

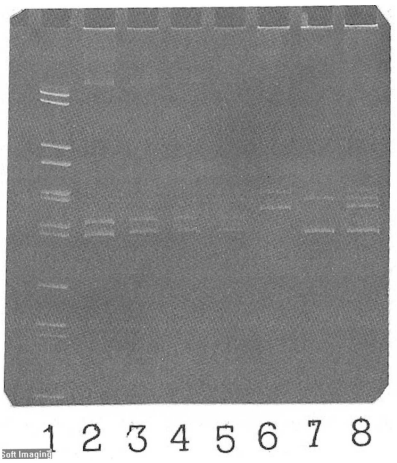

Fig. 2. Amplification of MCT 118 system. Lane 1: Marker VI (Boehringer); lane 2: control DNA from individual A; lanes 3, 4 and 5 : DNA extracted from semen stains of 50 µl, 25 µl and 10 µl; lane 6: DNA from individual B; lane 7: DNA from individual C; lane 8: extraction from stains made with a mixture B+C.

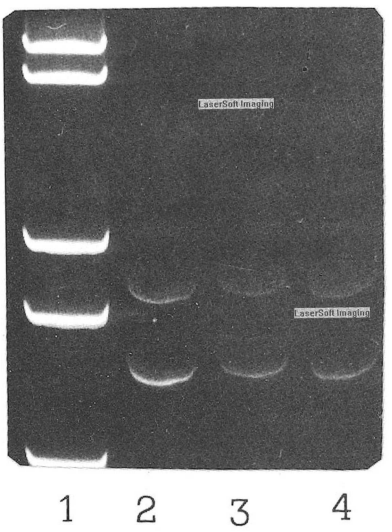

Fig.3. DNA extraction from cigarette butts. System Apo B. Lane 1: Marker VI (Boehringer); lane 2: blood DNA from individual A; lanes 3 and 4 : extractions from cigarette butts smoked from individual A.