A SIMPLE AND EFFICIENT NON-ORGANIC PROCEDURE FOR THE EXTRACTION OF DNA FROM EVIDENTIARY SAMPLES

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INTRODUCTION

The isolation of human genomic DNA from evidentiary samples is often time consuming and cumbersome. Existing methods typically involve the use of one or more of the following extraction techniques: digestion with a combination of Proteinase K and SDS or deproteination with organic reagents such as phenol and chloroform (Kanter 1986). Additional purification steps such as dialysis, precipitation with a saturated solution of NaCl and/or absolute ethanol are then required for enzymatic analysis (Miller 1988). The DNA isolation procedure described here is simple and efficient, eliminating the need for hazardous organic reagents or additional purification techniques.

This non-organic method for DNA isolation from evidentiary stains involves the incubation of the stained fabric with a high concentration of proteinase K at 65°C for 2 hours. It has previously been shown that proteinase K is at least seven fold more active on denatured protein and that prolonged incubation at high temperatures results in autoinactivation (Jeanpierre 1987). Following the 2 hour incubation the DNA extract can be used directly for restriction digestion.

This article describes the recovery of high molecular weight genomic DNA from dried blood stains and vaginal swabs and its subsequent use in determining RFLP patterns from stains as small as 1ul.

PREPARATION OF BLOOD STAINS AND VAGINAL SWABS

Blood stains were prepared from freshly drawn blood which was micropipetted onto white cotton cloth. Stain sizes of 1ul, 5ul, 10ul and 25ul were prepared, dried and stored at room temperature. Vaginal swabs were obtained from volunteers by swabbing the vagina approximately 10 minutes postcoitus. The cotton swabs were stored at -20°C until processing.

ISOLATION OF DNA

Blood Stains: Blood stained fabric was cut into small pieces (3x3mm) and placed into a 1.7ml microcentrifuge tube. Cells were lysed by the addition of 1ml ice cold cell lysis buffer (CLB, 0.32M sucrose, 10 mM Tris-HCL pH7.6, 5mM $MgCl_2$, 1% Triton X-100).

Advances in Forensic Haemogenetics 3
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The lysate was incubated on ice for 5 minutes (occasional vortex) and centrifuged 13,000 x g for 30 seconds. This process was repeated once more with CLB and then once with Protein Lysis Buffer (PLB, 10mM Tris-HCl pH8.0, 10mM Nacl, 10mM EDTA). Following the final centrifugation, residual PLB was removed from the tube by micropipetting. The fabric was then submerged in 40ul to 300ul (depending on size of fabric) of PLB containing 1mg/ml proteinase K. The tube was incubated at 65°C for 2 hours in a heatblock. Upon completion of the 2 hour incubation, the DNA lysate was removed from the fabric with a micropipet and transferred to a new tube.

Vaginal Swabs: The possibility of female cell contamination in samples obtained postcoitus necessitates a 2 step differential lysis to obtain DNA derived solely from sperm. The first lysis involves the isolation of female DNA obtained by incubating the fabric in a PLB/Proteinase K solution at 65°C for 2 hours as described above for bloodstains. Following the removal of the female DNA lysate, the fabric was washed 3 times with 1.5ml of PLB prewarmed to 65°C to remove trace amounts of female DNA from the fabric. At this point the sperm were still intact and could be lysed in a PLB/DTT/Proteinase K solution (PLB, 5mM DTT, 1mg/ml Proteinase K) at 65°C for 2 hours. Upon completion of the 2 hour incubation, the male DNA lysate was removed from the fabric.

RESULTS AND DISCUSSION

Several parameters are examined when DNA is recovered from evidentiary samples: 1) the quantity of DNA (yield), 2) the molecular weight of the DNA (integrity) and 3) the ability to digest the DNA with restriction enzymes. In order to obtain a reliable RFLP pattern from evidentiary samples an isolation procedure must be optimized for these three parameters.

Agarose gel electrophoresis indicates high molecular weight DNA was isolated from bloodstains 1-25ul in volume with total yields of approximately 25ng-1ug of DNA (Fig. 1A). Southern blot hybridization of this DNA indicates that the yield from all stains was sufficient for detection of RFLP patterns (Fig. 1B).

High molecular weight DNA was also obtained from the vaginal swab in both the female and male differential lysis steps (Fig. 2A). Southern blot hybridization demonstrates that the male lysate was free of contaminating female DNA. In contrast, the female lysate contained male DNA (Fig. 2B). This contamination was probably due to immature sperm which are less resistant to the female lysis step.

The recovery of DNA from biological samples requires that the DNA be of sufficient quantity and quality to perform RFLP analysis. Compared to existing methods, the DNA isolation technique described in this paper should significantly improve the recovery of DNA from evidentiary samples.

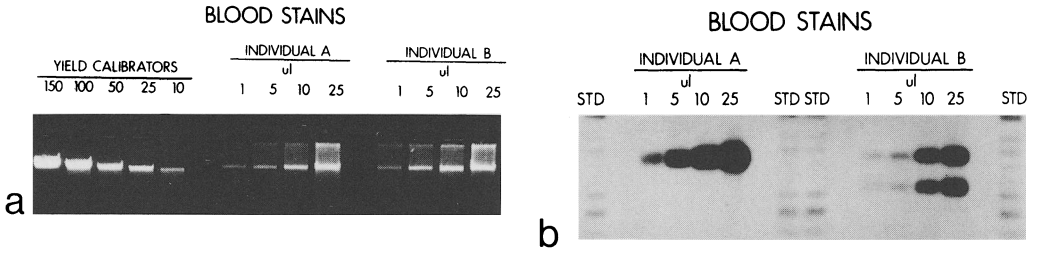

Fig. 1. (A) Isolation of high molecular weight DNA from bloodstains of two individuals. The DNA was fractionated in a 0.8% agarose gel containing ethidium bromide and photographed under UV light. The amount of sample loaded onto the gel ranged from 50% of the 1ul stain to 20% of the 5, 10 and 25 ul stains. (B) Southern blot analysis of DNA from bloodstains. The total amount of DNA obtained from each stain was digested with HaeIII and fractionated on a 1% agarose gel. Following Southern transfer, the blot was hybridized with an oligonucleotide probe localized to the human locus D2S44.

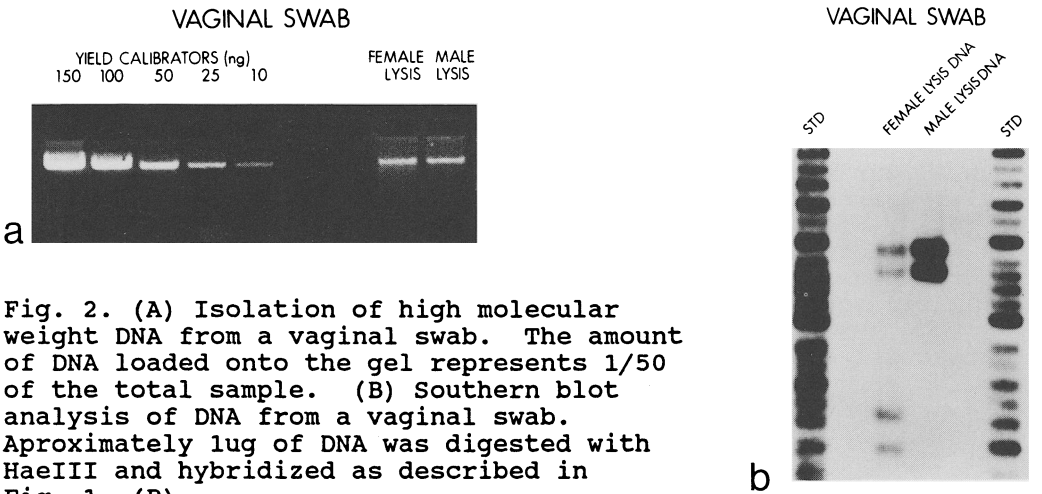

Fig. 2. (A) Isolation of high molecular weight DNA from a vaginal swab. The amount of DNA loaded onto the gel represents 1/50 of the total sample. (B) Southern blot analysis of DNA from a vaginal swab. Aproximately 1ug of DNA was digested with HaeIII and hybridized as described in Fig. 1. (B).

REFERENCES

Jeanpierre M (1987) A rapid method for the purification of DNA from blood. Nucl. Acids Res. 15:9611

Kanter E, Baird M, Shaler R, Balazs I (1986) Analysis of restriction fragment length polymorphisms in DNA recovered from dried bloodstains. J. For. Sci. 31:403-408

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from nucleated cells. Nucl. Acids Res. 16:1215