

The Isolation of DNA for Forensic PCR Analysis.
An Evaluation of Available Methodologies

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INTRODUCTION

PCR is generally considered to require less stringent sample purification than other analytical methods such as RFLP analysis. However, since it is intended to be used on particularly difficult samples (small, old, degraded,...), the success of the analysis will be highly dependent upon parameters like yield of the DNA isolation and presence of PCR inhibitors. For this reason, an evaluation of the merits of a few common or more recent DNA isolation methods was undertaken.

MATERIALS/METHODS

Stains

10µl stains were prepared from blood, sperm and saliva, diluted with NaCl 0.9% , giving stains containing from 0.1 to 10µl of the corresponding body fluid. These stains were allowed to dry and some were submitted to other conditions, giving rise to 4 types of stain treatment :

- A) drying at room temperature for 24h
- B) 5 days of direct exposure to summer sun (at 46° latitude north)
- C) 1 week heating at 75°C
- D) 1 week heating at 50°C in a water saturated atmosphere

DNA Extraction :

I. Chelex method (Singer-Sam et al. 1989) : add 500µl H₂O to the stain and incubate 30 min at room temperature. Spin 2 min at 15000 g and remove the supernatant {these first 2 steps are used only with blood and sperm stains}. Add 100µl 5% Chelex 100 (100-200 mesh, sodium form, Bio-Rad) {in the case of sperm, add also 1µl Prot.K 10 mg/ml and 4µl DTT 1M}. Incubate 30 min at 56°C (1h. for sperm). Vortex 5 sec. Boil for 8 min. Vortex 5 sec.

II. Methidium-Sepharose extraction (Harding et al. 1989) : to the stain, add 190µl 10mM Tris-HCl pH 8, 10mM EDTA, 100mM NaCl, 39mM DTT, 0.5% SDS, and 10µl prot.K 10 mg/ml. Incubate 1h. at 60°C. Punch a hole in the tube ; spin and recover the liquid phase. Add 50µl DNA capture reagent (Gibco-BRL) and agitate for 1h. Decant

the supernatant and wash the resin twice with 10mM Tris-HCl pH 7.5, 1mM EDTA, 100mM NaCl. Add NaOH 0.2M and agitate 10 min. Recover the eluted DNA and precipitate with ethanol. Dissolve the DNA in 100 μ l H₂O

III. Standard proteinase K digestion & phenol extraction

IV. Proteinase K digestion alone (Turck et al. 1990)

PCR Amplification of the D1S80 Locus

The efficiency of the DNA extraction was estimated by using 1/10 of the extract to amplify the locus D1S80 according to the method of Kasai (1990) with minor modifications. The PCR products were analysed on 2% agarose gel followed by staining with ethidium bromide.

RESULTS

In all the situations tested, the methods (II) and (IV) were at various degrees less efficient than the other two methods (fig. 1). Only methods (I) and (III) were retained for further testing.

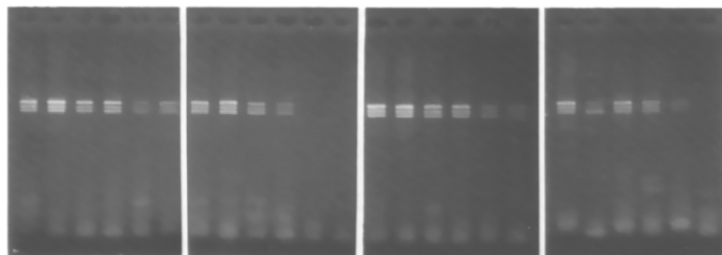
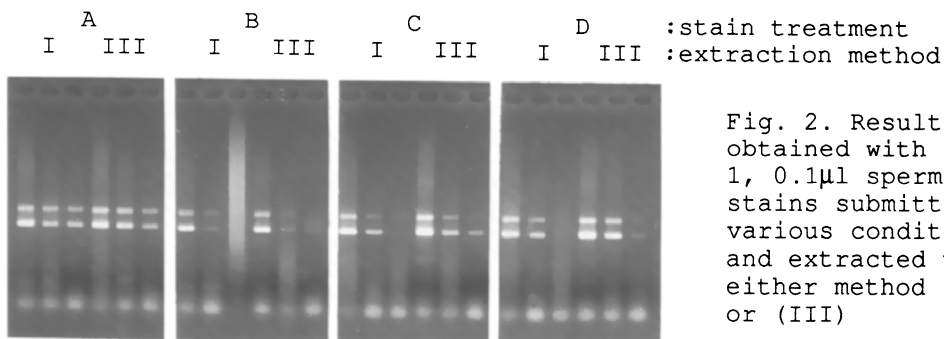


Fig. 1. Efficiency of the DNA extraction methods (I) to (IV) (from left to right) with fresh 10, 1, 0.1 μ l blood stains (in duplicate)

Of the four stain treatment conditions tested, the exposure to sun was the most detrimental. But methods (I) and (III) had the same efficiency in the DNA extraction of such mistreated stains (fig. 2).



This is true for most of the stain types tested, with two exceptions (fig. 3):

- blood stains on blue denim, where only method (I) gave a result;
- saliva stains on stamps where only method (III) was effective.

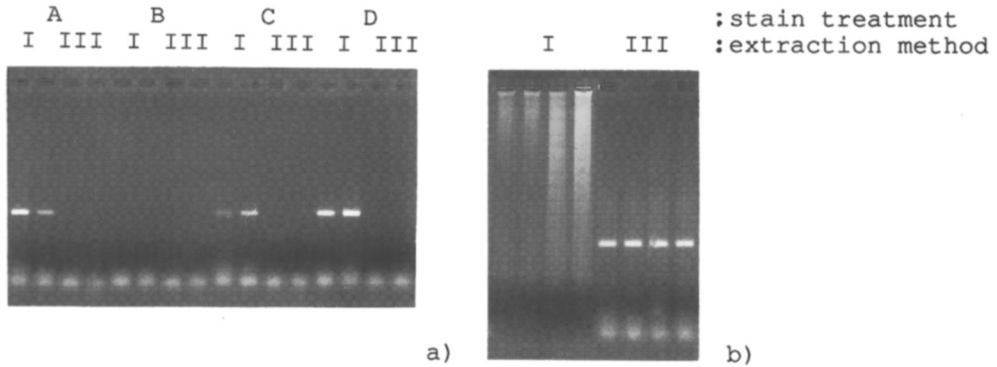


Fig. 3. Results obtained with a) 1 μ l blood stains on blue denim submitted to various conditions and extracted with either method (I) or (III) b) stamps pasted using 10 μ l saliva and extracted with either method (I) or (III).

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