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Research article

Automated DNA extraction from large volumes

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Abstract

Automation of DNA extraction with DNA-IQ™ (Promega Corporation), requires special adaptations when trace amounts of DNA must be recovered from substrates requiring large volumes of extraction buffer (e.g. clothing). We have developed a method consisting of *in situ* cell lysis with DNA extraction buffer (containing SDS or Sarkosyl, Proteinase K, DNA IQ lysis buffer) followed by batch DNA recovery through incubation with DNA-binding magnetic beads. Tests were performed with blood, semen, saliva and manipulated or worn objects on cotton, polyester and nylon substrates. The process was optimized for incubation time, temperature, detergent and Proteinase K concentrations. Non-probative forensic samples were tested and the procedure integrated into the robotic scripts.
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Keywords: DNA extraction; Large substrate; Automation; Magnetic bead

1. Introduction

Our laboratory has recently implemented automated DNA extraction using DNA-IQ™ and TECAN® robots. While most DNA-containing samples are easily amenable to automation, some still present a challenge to forensic laboratories faced with a broad range of substrates from which ever diminishing amounts of DNA must be recovered and analyzed for genetic fingerprinting purposes.

One such challenge is the recovery of DNA from substrates such as clothing from which only trace amounts of DNA are recovered. In such cases, it is necessary to remove relatively large tissue samples for analysis and to use 5 ml or more of extraction buffer. Since our automated procedure is set up to handle a maximum of 400 μl of extraction buffer per sample, it may require a dozen or more individual assays to purify the DNA with the added complication of having to pool the DNA-containing solutions eluted from the magnetic beads into a large and dilute final volume.

In order to reconcile automation and optimal DNA recovery from large substrates we have developed a method of direct cell lysis on the substrate followed by batch DNA concentration on magnetic beads in DNA-IQ™ extraction buffer.

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2. Batch lysis and DNA concentration method for large volumes

1. Approximately 25 cm^2 of substrate or tape lifts are cut up into 1 cm^2 pieces and placed in a 50 ml screw-cap tube containing 5 ml lysis buffer (10 mM Tris–HCl pH 8.0, 10 mM EDTA, 70 mM NaCl, 1% Sarkosyl, 0.04 mg/ml Proteinase K) and incubated overnight with rotation at 56 °C.

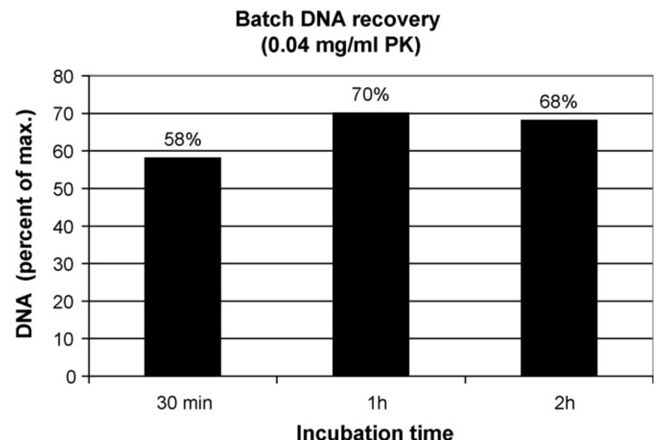

Fig. 1. Batch DNA extraction from 4 μl blood dried on cotton squares in the presence of 14 μl magnetic beads. Proteinase K is adjusted to 0.04 mg/ml.

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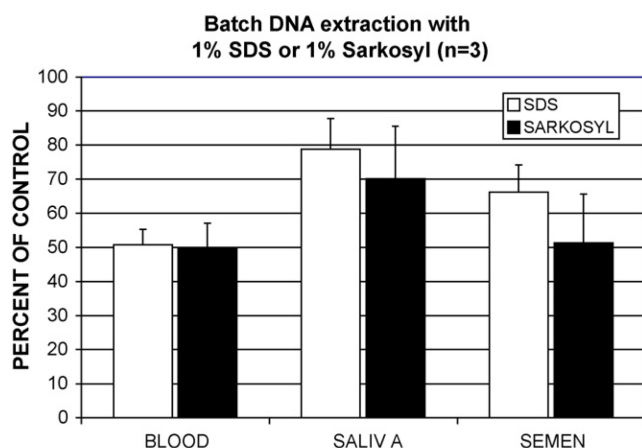

Fig. 2. Comparison of DNA recovered from three body fluids dried on cotton squares and extracted with 1% SDS (white) or 1% Sarkosyl (black) with the batch method. Control is the total amount of DNA extracted in small tubes and processed individually.

2. Tube contents are transfered to a spin-basket tube (Vectaspin 20™) and centrifuged at 3000 × *g* for 5 min. The tissue is discarded and the solution transfered to a clean 50 ml tube.
3. Two volumes of DNA-IQ™ extraction buffer are added together with 14 μl magnetic beads. The tubes are fixed to a rotary shaker and vigorously agitated at 120 rpm for 1 h at room temperature.
4. The tubes are placed in a large volume DNA-IQ™ magnetic rack, the supernatant discarded and the magnetic beads transfered to a mini-tube on the robot and the DNA-IQ™ protocol for DNA extraction resumed as per the manufacturer's instructions.

3. Conclusions

Batch extraction of DNA from large substrates allows recovery of 50–70% of the DNA obtained from cutting up the substrate into small pieces and processing each separately (Fig. 1). This greatly facilitates automation and in most cases is sufficient to obtain interpretable profiles. Another important advantage is the recovery of DNA in a small volume (25 μl) rather than in multiples of 25 μl which have to be reconcentrated before amplification.

Reducing Proteinase K from 0.5 mg/ml to 0.04 mg/ml nearly doubled DNA recovery, possibly due to reduced interference of Proteinase K with DNA binding to the beads. Replacement of SDS with Sarkosyl (Fig. 2) reduces foaming without significant loss of yield.

Conflict of interest

None.