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Optimization and validation of a fully automated silica-coated magnetic beads purification technology in forensics

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Abstract

Automated procedures for forensic DNA analyses are essential not only for large-throughput sample preparation, but are also needed to avoid errors during routine sample preparation. The most critical stage in PCR-based forensic analysis is DNA isolation, which should yield as much highly purified DNA as possible.

The extraction method used consists of pre-treatment of stains and samples, cell lysis using chaotropic reagents, binding of the DNA to silica-coated magnetic particles, followed by elution of the DNA.

Our work focuses mainly on sample preparation, obtaining the maximum possible amount of biological material from forensic samples, and the following cell lysis, to create a simple standardized lysis protocol suitable for nearly all forensic material.

After optimization and validation, the M-48 BioRobot® workstation has been used for more than 20,000 routine lab samples. There has been no evidence of cross contamination. Resulting DNA from as small as three nuclear cells yield reliable complete STR amplification profiles. The DNA remains stable after 2 years of storage.

Keywords: DNA extraction; Automated extraction; Short tandem repeat analysis; Real time PCR; Forensic DNA

1. Introduction

There are different methods described for DNA extraction from a wide range of specimens, commonly found at crime scenes. Only a few of the described methods are suitable for automation [1,2] and many of the steps involved possess a high risk of contamination. A lot of methods require centrifugation and solvent extraction steps [3,4], not suitable for automation. Most of the systems can only fulfil one of the requirements of DNA extraction, such as high DNA yield, rapidity of the method [5], high throughput or high quality of DNA [6,7]. Thus, the method of choice for many forensic samples was often the hazardous phenol chloroform extraction method applied under high safety measures often combined with other purification steps using membranes or columns.

Automated procedures for forensic DNA analyses are essential not only for large-throughput sample preparation, but are also needed to avoid errors during routine sample preparation. The most critical stage in PCR-based forensic analysis is DNA isolation, which should yield as much highly purified DNA as possible. We have searched for a DNA-preparation method with special precautions to avoid contamination events. A completely closed extraction robot with a drip tray and an UV sterilization system for decontamination between runs was selected.

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The extraction method described herein comprises the following steps: cell lysis using chaotropic reagents and binding of the DNA to silica-coated magnetic particles followed by elution of the DNA (Fig. 1). There are no manual steps between instrument loading and collecting the pure DNA sample.

Our work focuses mainly on sample pre-treatment, the only manual step, which we modified to obtain the maximum possible amount of biological material from forensic samples, and the subsequent cell lysis, to create a standardized lysis protocol suitable for nearly all forensic materials.

We have validated the precision, sensitivity and reliability of the presented extraction system.

2. Materials and methods

2.1. Sample pre-treatment

The optimization efforts of forensic DNA sample preparation were focused on sample pre-treatment and cell lysis steps to obtain the maximum yield of DNA from forensic specimen. Optimization has been performed for:

temperature: 56 and 90 °C;
time: 15 min–3 h;
addition of proteinase *K*: with/without;
lysis volume;
type of lysis buffer.

For the manual sample, pre-treatment different types of lysis buffer were tested: lysis buffer of the phenol/chloroform extraction method, lysis buffer without proteinase *K* and increasing amounts of Na_2EDTA. After pre-selection (data not shown), the following lysis buffers were validated:

(1) enzymatic lysis buffer (10 mM Tris–HCl, pH 7.4, 400 mM NaCl, 2 mM Na_2EDTA, pH 8.1, 1% SDS and 667 μg/ml proteinase *K*);
(2) detergent lysis buffer (50 mM Tris–HCl, pH 7.4, 100 mM NaCl, 100 mM Na_2EDTA, pH 8.1, 1% SDS);
(3) TN_{CA}-buffer (10 mM Tris–HCl, pH 8.0, 100 mM NaCl, 1 mM $CaCl_2$, 2% SDS, 39 mM DTT, 250 μg/ml proteinase *K*) [8].

Details of the resulting method for each sample type and quantitation results are shown in Table 1.

In the following, a protocol is presented for the pre-treatment of forensic specimen:

- The forensic specimen (swab, cotton substrates, etc.) is placed in a 1.5-ml tube.

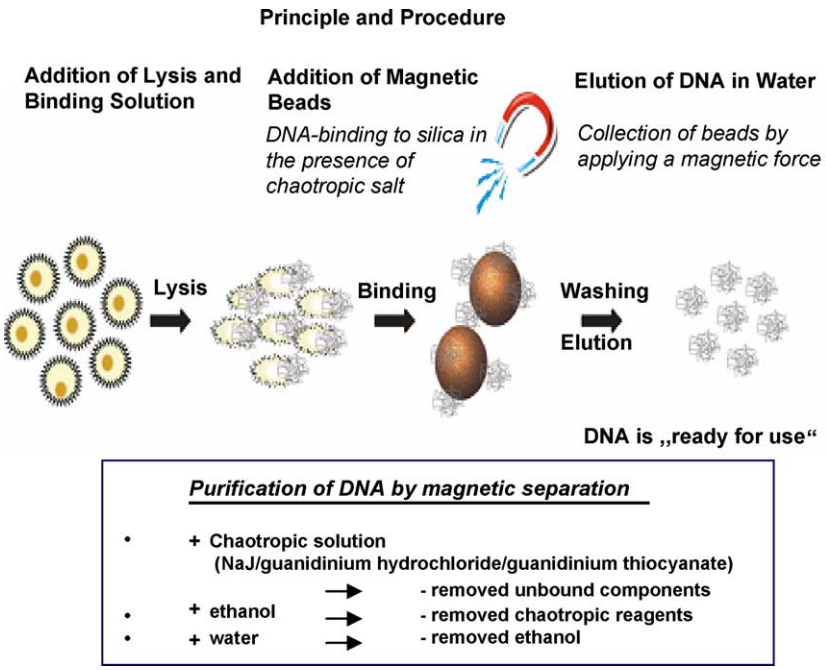

Fig. 1. Principle and procedure of the DNA extraction method over silica-coated magnetic particles according to the manufactures manual (GENOVISION, Wien, Austria; Qiagen, Hilden, Germany). Cells are lysed in the presence of a chaotropic solution. Silica-coated magnetic particles are added. The DNA binds to the silica-coated magnetic particles. Two washing steps follow the first with a chaotropic salt solution, to remove non-bound matter, the second with ethanol, to remove salt. A short rinse with water removes the ethanol, and finally the purified DNA is eluted in water ready for use in down stream applications.