promega
Application Notes

Extraction and Isolation of DNA from Blood Cards and Buccal Swabs in a 96-well Format

By Paraj Mandrekar, Laura Flanagan, and Allan Tereba • Promega Corporation

Summary

New protocols were developed to extract and purify DNA from commercially available blood cards and buccal swabs in a 96 well format using the DNA IQ™ System and the Beckman-Coulter Biomek® 2000 Laboratory Automation Workstation. The high throughput extraction technique can also be used with manual DNA IQ™ purification.

Introduction

The DNA IQ™ System (Cat.# DC6700), uses a limiting amount of a novel paramagnetic particle to purify a consistent amount of DNA from forensic database samples. In the low throughput manual format, efficient extraction of DNA from solid supports was accomplished with a 30 minute incubation in Lysis Buffer at 95°C and centrifugation to force the DNA out of the matrix. This treatment was necessary for improved consistency with some substrates such as FTA® paper. While this extraction step is efficient, it is not easily automated.

In developing a high throughput extraction method using a 96-well format, we concentrated on a simple method that required minimal manual intervention, was amenable to different sample types and could be easily configured as a front end module of our Biomek® 2000 DNA IQ™ purification program. This method eliminates the centrifugation step to force DNA out of the support, resulting in significantly reduced yields. We compensated for this by using larger blood card punches and whole buccal swabs. The use of whole swabs has the advantage that minimal manipulation is needed. Elimination of the centrifugation step necessitated a longer incubation period in lysis buffer with vigorous vortexing before and after to obtain consistent yields. This extended process should have a minimal effect on work flow as multiple plates can be processed at one time, and the samples can be stored overnight at room temperature before processing.

Materials and Methods

Sample preparation:

Blood card samples were prepared by saturating FTA® or Schleicher and Schuell (S & S) 903 paper with liquid blood and drying at room temperature. Cotton and CEP paper buccal swabs were prepared from various individuals and dried overnight at room temperature.

Sample incubation:

Blood card punches (1/8" diameter) or whole cotton or paper buccal swabs were placed in the wells of a Beckman 96-well plate (2.2ml volume). For swabs, the shaft was either broken off near the head (cotton) or the head ejected with the plunger (paper). DNA IQ™ Lysis Buffer was added a volume of 300µl (punches), 500µl (cotton swab) or 1ml (paper swab).

The plates were sealed with a plate seal (Robbins CycleFoil® Storage Plate Sealer, Cat.# 1044-39-3) and vortex mixed for 30 seconds at high speed, holding the plates level throughout the vortexing process. The sealed deep well plate was then incubated at 90°C for 2 hours in a covered waterbath. A high temperature oven can also be used. After the 2 hour incubation, the plates were again vortexed for 30 seconds and then centrifuged for 2 minutes. This brief centrifugation is necessary to eliminate cross-contamination. The seal was removed and the plate was placed on a Biomek® 2000 Laboratory Automation Workstation for DNA purification using the DNA IQ™ System and a Bioworks™ program designed for these sample types.

Automated purification:

A Bioworks™ method exists for DNA isolation from aqueous samples on the Beckman-Coulter Biomek® 2000 Workstation and is currently being used in several forensic laboratories[a]. The beginning of this method was modified to allow DNA purification from blood cards and buccal swabs. This modification transfers 300µl of the DNA-containing sample to a clean deep well plate, eliminating the need to manually remove the swab or punch. DNA IQ™ Resin is added, and the solution is mixed and incubated to allow the DNA to bind the Resin. Subsequent steps follow our program for aqueous samples. This process minimizes variations in DNA purification from different sample types.

To test this format with FTA® blood cards, samples from two individuals were run in a checkerboard pattern. In addition, cotton buccal swabs obtained from five different individuals were processed. The DNA was eluted in 100µl of DNA IQ™ Elution Buffer and amplified with a TH01 monoplex (blood card punches) or the PowerPlex® 16 System (buccal swabs) and then analyzed on an ABI PRISM® 310 Genetic Analyzer.

Results

Purification of DNA from FTA® blood cards

The results for the purification and amplification of 24 samples on the Biomek® 2000 Workstation are shown in Figure 1. The average amount of DNA recovered was 90ng ± 7ng standard deviation. There was no significant variation between samples for the two individuals and no cross contamination was observed (the two individuals had different TH01 alleles). These results demonstrate a reproducible yield, which allows consistent amplification without a quantitation step. Reducing the incubation temperature, time or vortexing rate resulted in a reduction of recovered DNA and more variable results. S&S 903 blood cards gave similar results.

Excluding the placement of samples, the entire process requires approximately 3.5 hours for 24 samples (about 5 hours for 88 samples). The hands on time (excluding sample placement) is only approximately 20 minutes regardless of sample number.

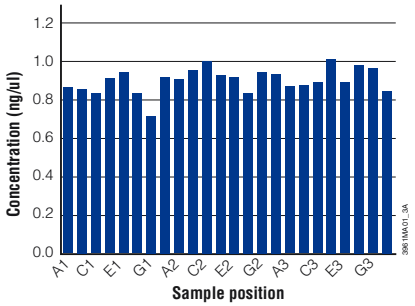

Figure 1: DNA purification from FTA® blood cards on the Biomek® 2000. Twenty-four individual 1/8" diameter punches from blood saturated FTA® paper were preprocessed at 90°C for 2 hours prior to DNA purification on the Biomek® 2000. An equal volume of purified DNA was amplified with a TH01 monoplex and analyzed on an ABI PRISM® 310 Genetic Analyzer. Resulting fluorescent intensities were compared with a standard curve generated by amplifying known concentrations of 9947A DNA to estimate the concentration of DNA in each sample.

Robotic DNA isolation from cotton buccal swabs

The use of buccal swabs for database and reference samples is non-invasive, has a lower potential for transmitting diseases and does not require a specialist to obtain the sample. Unfortunately, this sample type provides the greatest variation in DNA yield. These variations are the result of differences in swabbing technique, differences in how individuals shed cells and most importantly, how samples are stored. We have found that swabs that are not thoroughly dried and then stored at room temperature for more than a month give low and variable yields. This is probably due to the growth of microbes, which degrade human DNA and eventually overwhelm the human DNA that is left. In our experiments, we have thoroughly dried swabs prior to storage at room temperature.

Figure 2 shows banding patterns of PowerPlex® 16 amplification reactions from 6 different cotton swabs from 5 individuals using a set volume of DNA in the amplification reactions. All DNAs were easily genotyped. The results obtained from CEP paper swabs were similar.

In preliminary experiments with The Bode Technology Group's new buccal swab system, we observed lower yields and more variability using 1/8 inch punches than with cotton swabs. Increasing the size of the punch to 1/4 inch or reducing the amount of DNA IQ™ Resin during purification from 7µl to 3.5µl resulted in variation between samples of about 2 fold, consistent with cotton and paper swabs.

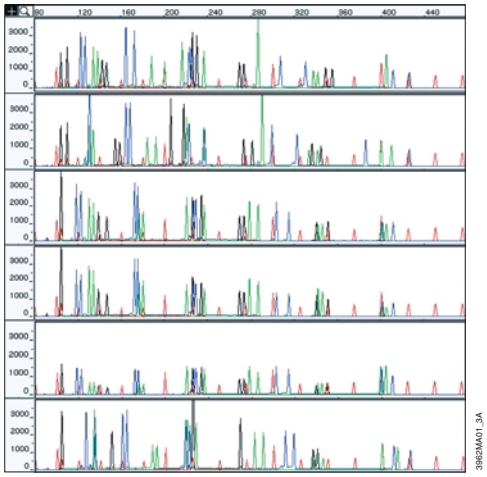

Figure 2: Amplification of purified DNA from cotton buccal swabs with the PowerPlex® 16 System. DNA from 6 cotton buccal swabs was purified using DNA IQ™ reagents on a Biomek® 2000. Of the 100µl of eluted DNA, 2µl was amplified using the PowerPlex® 16 System and analyzed on an ABI PRISM® 310 Genetic Analyzer.

Conclusion

A simple 96-well format that requires minimal hands on time has been developed to extract DNA from blood cards and buccal swabs. After the incubation of samples, the plate containing the blood card punches or swabs can be placed on a Beckman-Coulter Biomek® 2000 Workstation for a hands-off purification of DNA using DNA IQ™ reagents. For medium-throughput, extraction of DNA from blood card punches and swabs can be done in a plate or individual tubes followed by manual purification of DNA with DNA IQ™ reagents.

(A) Greenspoon, S. and Ban, J. (2002) Robotic extraction of mock sexual assault samples using the Biomek® 2000 and the DNA IQ™ system. *Profiles in DNA* **5**, 3-5.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601

Printed in USA 2/03
10376-AN-GI
Part #AN102

Promega
www.promega.com