AUTOMATION OF *IN SITU* DNA SAMPLE PREPARATION FOR PCR USING THE FTA™ DNA COLLECTION SYSTEM AND THE ROSYS LABORATORY WORKSTATION

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INTRODUCTION

The development of dried blood stain technology has greatly facilitated the collection, transport, and isolation of DNA from blood samples. Blood stain cards are currently being used in clinical screening programs for Cystic Fibrosis (Audrezet and Costes, 1993; Raskin, 1992) and Human Immunodeficiency Virus (HIV) (Nyambi, 1994; Cassol, 1991). Within the forensic community, blood stain cards are being used to collect DNA samples from convicted felons and/or sex offenders, as military reference samples (Williams et al., 1994), and for parentage testing. Current blood stain technology, which is based on the use of filter paper cards, has provided a simple system for the collection, transport, and storage of whole blood DNA samples. However, the DNA must be extracted from the blood stain card prior to amplification and analysis.

The FTA DNA collection system provides a new and integrated approach to blood stain technology. The FTA system begins with a blood stain card which is chemically impregnated to kill any existing pathogens in the collected blood sample and to protect against bacterial and environmental degradation of the DNA.

Current protocols for PCR based analysis of DNA from blood stain cards require that the DNA be extracted from the card prior to amplification, or the protocols contain steps which liberate the DNA from the card as part of the amplification process (McCabe 1987; Carducci et al., 1992; Gregory et al., 1995; Harvey et al., 1995). Methods for the micro-extraction of DNA, particularly those using ion exchange resins or cards treated with chaotropic agents, are time consuming and/or labor intensive. These extraction methods are not amenable to automation since they require vortexing, centrifugation, and in some cases boiling of the sample. To overcome these obstacles to high through-put DNA sample preparation, a novel, integrated system for DNA collection and purification has been developed. This new method uses a single reagent, is fast, truly automatable, involves no manipulation of the sample during processing, and permits amplification of the sample directly from the card (Figure 1.). The Rosys laboratory workstation used in this method can also be used to set-up PCR reactions following sample preparation.

MATERIALS AND METHODS

FTA and S&S 903 blood stain cards were spotted with anticoagulated whole blood and allowed to dry. The blood stain cards were then placed in vapor barrier bags and stored at -20°C. For sample processing, a $2mm^2$ or 3mm circular punch was taken from either the FTA or S&S card. Samples were then placed into tubes and/or 96-well plates as appropriate for the experiments.

In Situ Sample Preparation

In Situ sample preparation was performed using various reagent protocols: Phenol/Chloroform/Isoamyl Alcohol; Proteinase K; Wizard™ Genomic DNA Purification Kit (Promega Inc., Madison, WI); and One-Step DNA purification reagent (Fitzco, Inc. Maple Plain, MN) and the Rosys 3300 robotic workstation (Rosys, Wilmington, DE).

Phenol/Chloroform/Isoamyl Alcohol

200 µl of buffered phenol/chloroform/isoamyl alcohol was added to each well and the plate was incubated for 30 minutes at 50°C. The samples were then washed once with 200 µl of the phenol solution, and three times with 200 µl of isopropanol/0.1M potassium acetate (75%/25%, v/v, pH 7.8). Samples were then incubated for 20 minutes with 200 µl of isopropanol/0.01M magnesium acetate (75%/25%, v/v, pH 7.8) at room temperature, washed with 100 µl of pure isopropanol, and dried for 10 minutes at 50°C.

Proteinase K

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200 µl of cell lysis buffer was added to each well and the plate was incubated for 15 minutes at 27°C. Incubation in fresh cell lysis buffer was repeated for a total of three times. Samples were then incubated for two hours at 60°C in 200 µl of Proteinase K digestion buffer (10 mM Tris-HCL, 400 mM NaCl, 2 mM Na_2EDTA, 1% SDS, Proteinase K (20 mg/ml) and 0.1% B-mercaptoethanol). Samples were then washed three times with 200 µl of isopropanol/0.1M potassium acetate, incubated for twenty minutes in 200 µl of isopropanol/0.01M magnesium acetate at RT (22°C), washed with 200 µl of pure isopropanol, and dried for 10 minutes at 50°C.

Wizard Genomic Purification Kit
200 µl of cell lysis buffer was added to each well and the plate was incubated for 15 minutes at 27°C. Incubation in fresh cell lysis buffer was repeated for a total of two times. Samples were then incubated for 15 minutes in 200 µl of nuclei lysis buffer at 27°C, washed with 200 µl of isopropanol/0.01M magnesium acetate, 200 µl of pure isopropanol, and dried for 10 minutes at 50°C.

One-Step™ DNA Purification
200 µl of One-Step reagent was added to each well and the plate was incubated for 15 minutes at 27°C. Incubation in fresh One-Step was repeated for a total of three times. Samples were then washed with 200 µl of pure isopropanol and dried for 10 minutes at 50°C.

Samples from this study were analyzed using the DQ-α typing system and/or a STR system (D1S80, F13B, or CSF1PO).

PCR amplification and analysis
All PCR reactions were carried out in a Perkin Elmer DNA Thermal Cycler (Foster City, CA). The reverse dot blot system , Amplitype HLA DQ-α (Perkin Elmer, Foster City, CA) was amplified and analyzed in accordance with the manufacturer's instructions. STR systems (F13B and CSF1PO) (Promega, Madison, WI) and D1S80 (Perkin Elmer, Foster City, CA) were amplified in accordance with the manufactures instructions. Amplification products were analyzed on yield gels, 2% agarose containing 0.125 µg/ml ethidium bromide. The amplification products were run at 80 volts for 45 minutes and photographed on a UV transilluminator.

Vertical gel electrophoresis was performed using 40 µm, 6% polyacrylamide denaturing gels. Samples, ladders, and sizing markers were loaded using STR 2X Loading solution (Promega, Madison, WI) and run at a constant current of 1.3watts/cm. Following electrophoresis, the samples were silver stained using the Promega DNA Silver Staining System.

Comparison of FTA and S&S 903 Blood Stain Cards
3 mm punches of spotted FTA and S&S 903 cards were placed into a 96-well plate and processed using One-Step DNA purification reagent. Following sample preparation, DNA was amplified using the D1S80 primer set according to manufacturer's guidelines. Amplification products were analyzed on a 2% agarose gel as described.

Contamination Study
Samples of spotted FTA cards were processed in two ways: First, samples of two individuals were placed in a 96-well plate, with every fourth well containing both of the samples. Following processing with One-Step DNA reagent, the samples were amplified individually using the Amplitype DQ-α typing system. Second, six samples each from two individuals were placed in the same tube (total of twelve punches), processed using One-Step DNA reagent and amplified individually using the D1S80 typing system.

SUMMARY

1. An evaluation of various reagent systems for the automated pre-amplification sample preparation of DNA from FTA blood stain cards was performed. Fitzpak One-Step™ DNA purification reagent proved to be more effective than phenol/chloroform/isoamyl alcohol or the Wizard reagent system. Amplification of DNA following sample preparation with One-Step resulted in robust amplification products and typable results. the proteinase K protocol used in this study was not effective and resulted in poor amplification signal.

2. An evaluation of amplification from FTA and S&S 903 blood stain cards was performed. Following sample preparation with One-Step DNA purification reagent, the FTA blood stain card proved to be a superior matrix for the direct amplification of DNA and routinely produced typable results.

3. Cross-contamination studies indicate that there is no migration of the DNA during any of the sample preparation protocols. The FTA matrix tightly binds the DNA while releasing PCR inhibitors and cellular debris.

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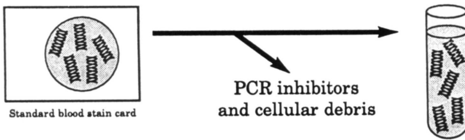


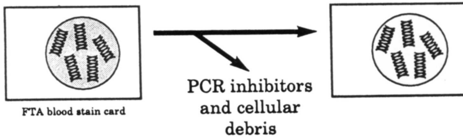
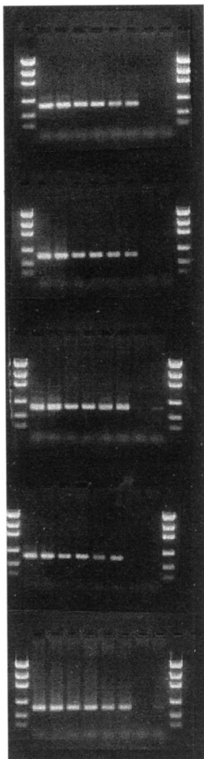
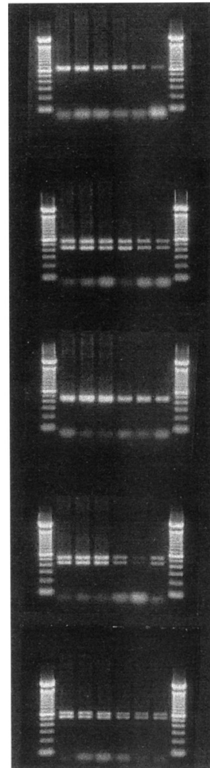


Figure 1. Schematic diagram of traditional DNA extractions from blood stain cards and in situ DNA purification using the FTA blood stain card. In traditional extraction procedures, which can include vortexing, centrifugation, and boiling, the DNA is removed from the card, purified from PCR inhibitors, and then concentrated in a tube. The FTA DNA system removes PCR inhibitors from the card and leaves the purified DNA on the card. This DNA is now ready for PCR amplification.





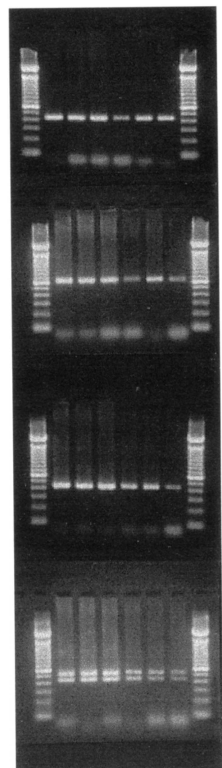


Figure 2. Comparison of the four DNA purification methods. Samples of the FTA bloodstain cards were purified using one of the four reagent systems: phenol/chloroform/isoamyl alcohol, poteinase K, Wizard genomic DNA reagents, or One-Step. DNA samples were amplified using the STR CSF1PO amplification system and analyzed on 2% agarose yield gels. Lanes 2-3 = One-Step, Lanes 4-5 = Phenol, Lanes 6-7 = Wizard, Lanes 8-9 = Proteinase K. Lanes 1 and 10 = Markers.

Figure 3. Comparison of amplification from FTA and S&S 903 blood stain cards. Whole blood samples from 9 individuals were spotted onto both FTA and S&S 903 blood stain cards. Following purification of the DNA with One-Step DNA reagent, the samples were amplified using the D1S80 amplification system. Following amplification, the samples were analyzed using 2% agarose yield gels. Lanes 1 & 8 = 100 Bp ladder; Lanes 2-4 = FTA; Lanes 5-7 = S&S903

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4. The Rosys Workstation is an integral part of the FTA DNA system and can be programmed to perform not only DNA sample preparation, but PCR set-up in the 96-well plate format or standard reaction tube format.

5. The FTA DNA system is a safe, single reagent, automated procedure which begins with sample collection and finishes with high quality amplification products. This system is designed for high-throughput processing of DNA, and can provide hundreds of samples per day.

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