

Extraction of High Quality DNA from Biological Materials using the AutoMate Express™ Forensic DNA Extraction System and PrepFiler Express™ Forensic DNA Extraction Kits

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ABSTRACT

The PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits and the AutoMate Express™ DNA Extraction System have been developed to meet all criteria for forensic applications. These kits and automated extraction platform enable the isolation of genomic DNA simultaneously from up to 13 forensic biological samples. The extracted DNA is free of PCR inhibitors and ready for downstream applications such as real-time qPCR and STR genotyping. The reagents are packaged in sealed cartridges for processing individual samples minimizing risk of cross contamination and achieving consistent DNA yields. Walk-away operation increases both the efficiency of trained forensic analysts and the throughput of forensic labs. The protocols are optimized for extraction of DNA from a variety of sample types including: blood stains on variety of fabrics, FTA® paper, samples spiked containing PCR inhibitors, saliva on swabs, semen on cotton fabric, bones, hair roots, tape lifts, and touch evidence samples. DNA obtained from these samples was free of detectable PCR inhibitors and resulting short tandem repeat (STR) profiles were complete, conclusive, and devoid of PCR artifacts.

INTRODUCTION

Forensic analysts encounter a variety of biological samples including deposits of blood, saliva, and semen evidence on a variety of substrates, as well as hair, bones, teeth, finger nail scrapings, and "touch" evidence - any of which may have been exposed to a range of environmental assaults or contaminants. The genomic DNA contained in these samples is associated with many cellular components and macromolecules that compact and protect the DNA *in vivo*. If not removed during the DNA extraction procedure, these accessory factors can interfere with the downstream processes of DNA analysis, namely, PCR. Therefore, it is important that the procedure used to isolate genomic DNA is efficient and delivers DNA in a highly purified form. It is also desirable to have an extraction methodology that enables quantitative recovery of DNA from small quantities of starting material and yields DNA in a highly concentrated form, so that volumes used for PCR can be minimized. The isolation procedure must also remove the vast majority of PCR inhibitors and, moreover, work for most specimen types. Finally, all extraction reagents and steps of the protocol should be amenable to automation. The PrepFiler Express™ and Express BTA™ Forensic DNA Extraction kits have been developed to meet the needs of a forensic DNA extraction chemistry. The PrepFiler Express™ BTA kit has been optimized for the extraction of bones, teeth, and adhesive containing substrates, while the PrepFiler Express™ kit can be used for the majority of other forensic case-type samples.

MATERIALS AND METHODS

DNA extractions were performed using PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction kits operated with AutoMate Express™ System (Figure 1). When processing samples with PrepFiler Express™ and PrepFiler Express BTA™ kits, newly developed PrepFiler LySep™ columns (Figure 2) were used to provide separation of lysate from sample solids. Additional materials, kits, analytical instruments, software used and methods of analysis are described previously [Brevnov, M.G. et. al. 2009. Validation of the PrepFiler™ forensic DNA extraction kit for extraction of genomic DNA from biological samples. J Forensic Sci. 54: 599-607]. For processing of samples with the PrepFiler Express™ kits, 500 µL of PrepFiler™ Lysis Buffer was used for lysis of each sample. The lysis was performed by incubation at 70°C with shaking at 750 rpm for 40 min. For processing of samples with the PrepFiler Express BTA™ kit, 230 µL of PrepFiler BTA™ lysis solution (contains PrepFiler BTA™ Lysis Buffer, Proteinase K and DTT) was used for lysis of each sample. The lysis was performed by incubation at 56°C with shaking at 1100 rpm for 2 h for bone and tooth samples. Human DNA quantities were determined using the Quantifiler® Duo DNA Quantification Kit (Figures 3A and 3B). STR profiles were generated using the Identifier® Kit (Figures 4 and 5).

Substrates	Biological tissues
Cotton	Blood
Denim	Saliva
Cotton tipped applicator	Epithelial cells
Rayon	Semen
Wool	Hair
Filter paper	Bone
FTA paper	Tooth
Plastic	
Adhesive tape	
Cigarette butt	

Table 1. Types of substrates and biological tissues tested on AutoMate Express™ System using PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction kits.



Figure 1. Bench top AutoMate Express™ System using PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction kits.



Figure 2. PrepFiler LySep™ column was developed to provide effective one-step lysis and separation of large size particulate from lysate without any manual transfer steps.

RESULTS

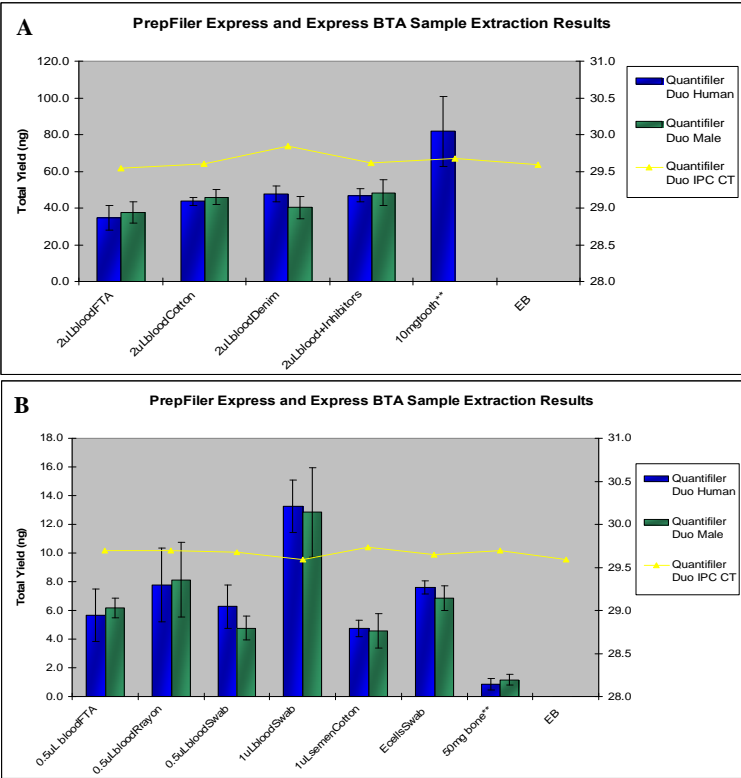
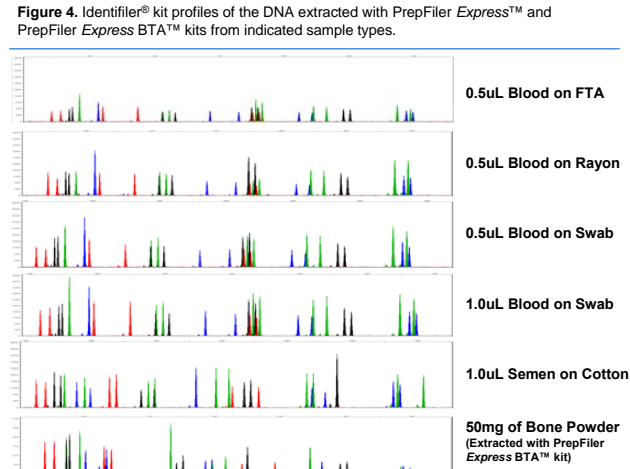
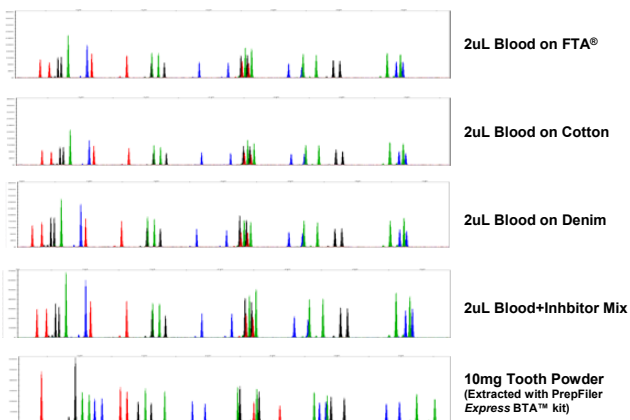


Figure 3. Results of DNA extraction from variety of samples using PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits. **Samples:** Graph A: 2uL blood dried on FTA® paper, 2uL blood dried on cotton cloth, 2uL blood dried on blue denim, 2uL blood + inhibitor mix* dried on cotton cloth, and 10mg of tooth powder**. Graph B: 0.5uL blood dried on FTA®, Rayon, and Cotton Swab, 1.0uL semen dried on cotton cloth, E-cell suspension dried on cotton swab, and 50mg bone powder**.

*Inhibitor mixture contains hematin, humic acid, Indigo and urban dust extract

**50mg Bone Powder and 10 mg Tooth Powder extracted with PrepFiler Express BTA™ kit



CONCLUSIONS

The PrepFiler Express™ and PrepFiler Express BTA™ kits operated with the AutoMate Express™ System demonstrated high efficiency of DNA isolation from a broad variety of forensic samples. The PrepFiler Express™ kit enables extraction of the majority of case-type forensic samples and the PrepFiler Express BTA™ kits extracts DNA from calcified tissues and adhesives. Simplified off-line lysis with the PrepFiler LySep™ column enables one-step lysate and substrate separation eliminating the need for manual sample and tube transfers. Sealed cartridge-based DNA isolation on the AutoMate Express™ System reduces contamination events and manual extraction steps. Isolated DNA was free of PCR inhibitors and suitable for downstream applications such as qPCR and STR PCR.

ACKNOWLEDGEMENTS

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