

DNA extraction

PrepFiler *Express* and AutoMate *Express* DNA extraction systems

Introduction

Forensic samples are among the most difficult specimens to process because they are often limited in quantity, may be environmentally exposed, and may be present on substrates containing PCR inhibitors. The quantity and quality of genomic DNA extracted and purified from a forensic sample are directly correlated to the success of downstream analysis.

The Applied Biosystems™ AutoMate *Express*™ Forensic DNA Extraction System is an easy-to-use, robust, benchtop instrument that offers maximum flexibility and utilizes the Applied Biosystems™ PrepFiler *Express*™ and PrepFiler *Express* BTA™ (bone, tooth, and adhesive) chemistries packaged in prefilled, foil-sealed cartridges (Figure 1). The PrepFiler *Express* kits were specifically designed to improve the quantity and quality of DNA isolated from forensic samples, increasing the ability to obtain maximum information from downstream short tandem repeat (STR) analysis.

- Designed to maximize results from varying input types, ranging from high-quantity reference samples to compromised crime scene samples
- Maximized productivity and minimized risk of contamination with reduced hands-on time and easy setup for faster time-to-result
- Optimized for the latest Applied Biosystems™ quantification and STR kits to help improve downstream results



Figure 1. The AutoMate *Express* system simultaneously processes 1–13 samples in a single run utilizing the PrepFiler *Express* and PrepFiler *Express* BTA chemistries with prevalidated and preprogrammed instrument protocols.

Flexible, automated solution

The AutoMate Express system offers the greatest range of elution volumes, maximizes the ability to obtain interpretable STR results, and allows for the flexibility of choosing an elution volume (Figure 2). Higher elution volume methods may be better suited for samples expecting to have high quantities of DNA (reference samples); whereas lower elution volume methods may be better suited for use with lower-quantity samples (touch samples). We have demonstrated that use of the 20 μL elution volume method reduces, or even eliminates, the need to perform subsequent sample concentration protocols using Microcon™ centrifugal filters (Merck) or Thermo Scientific™ Savant™ SpeedVac™ concentrators—leading to shorter time-to-results, and reduced costs and risk of contamination (Figure 3).

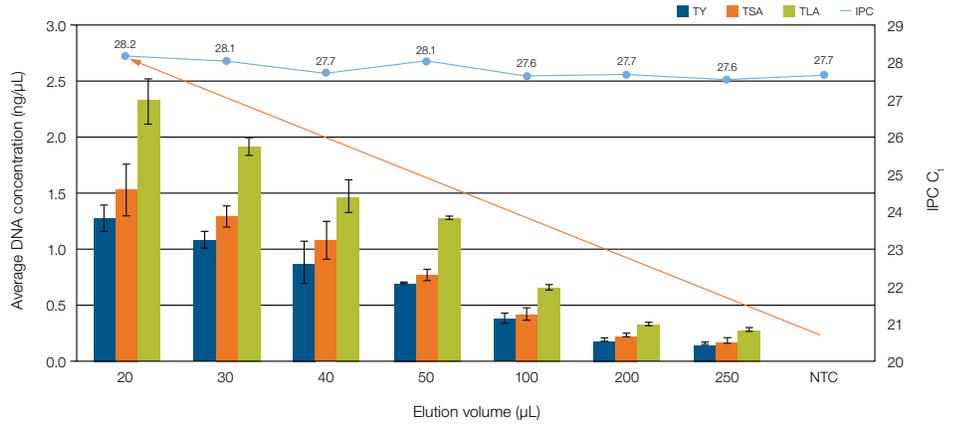


Figure 2. A range of elution volumes was tested using the AutoMate Express system. DNA was extracted from the same blood sample using variable elution volume methods on the AutoMate Express instrument and quantified using the Applied Biosystems™ Quantifiler™ Trio DNA Quantification Kit. The data for the Y chromosomal target (TY), small autosomal target (TSA), and large autosomal target (TLA) demonstrate a linear correlation between elution volume and average DNA concentration (orange arrow), while the internal positive control threshold cycle (IPC Ct) value remains consistent. Similar linearity was observed with buccal samples and samples extracted with the PrepFiler Express BTA protocol (not shown).

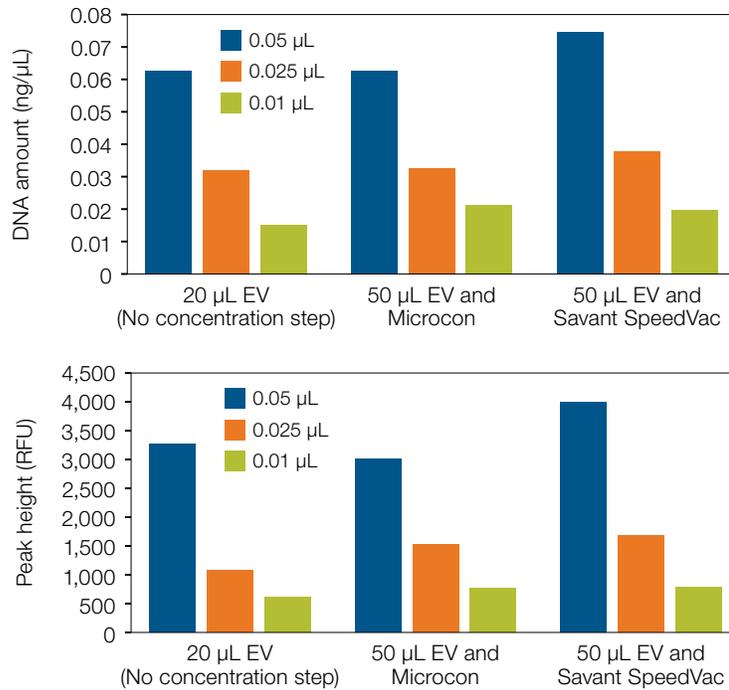


Figure 3. Comparison of quantitation results with and without sample concentration step. The same blood sample was processed in duplicate at three different concentrations (0.01 μL , 0.025 μL , and 0.05 μL) using the 20 μL variable elution volume (EV) method on the AutoMate Express instrument: not concentrated, the 50 μL standard EV on the AutoMate Express instrument; concentrated using a Microcon filtration device, and the 50 μL standard EV on the AutoMate Express instrument; concentrated using a Savant SpeedVac concentrator. The samples were quantified with the Quantifiler Trio kit and amplified with the Applied Biosystems™ GlobalFiler™ PCR Amplification Kit. Consistent quantitation results were obtained for the three methods, and the 20 μL elution protocol generated samples with comparable peak heights to those produced from the 50 μL elution protocols followed by concentration of samples.

Streamlined and easy-to-use DNA extraction system

The AutoMate *Express* system enables workflow flexibility by offering the capability to customize the number of samples for extraction on each run (1–13 samples capability), in comparison to a larger robotic platform suited for higher-throughput sample processing. This functionality allows for more control of the extraction workflow, a manageable sample size, and more efficient use of reagents. The ease of instrument setup, minimal handling of sample tubes, and the short run time make the AutoMate *Express* system an ideal instrument for analysts to quickly perform unscheduled extractions for expedited cases.

The PrepFiler *Express* and PrepFiler *Express* BTA kits feature consumables designed to streamline DNA extraction while minimizing contamination (Figure 4). From sample lysis and substrate removal in a single tube to easy loading of the

sample into the AutoMate *Express* instrument, the simplified workflow requires minimal effort and enables maximum results.

The PrepFiler *Express* kits include the innovative Applied Biosystems™ PrepFiler™ LySep™ Column that streamlines substrate separation during the extraction process. PrepFiler *Express* Lysis Buffer or PrepFiler *Express* BTA Lysis Buffer is added directly into the PrepFiler LySep Column along with the sample for incubation. Next, the PrepFiler LySep Column is centrifuged at a high speed, which permits lysate to flow through the proprietary burstable membrane while the substrate remains behind in the column. The sample tube is then loaded directly into the AutoMate *Express* instrument, eliminating the manual lysis and substrate transfer steps, which helps save time and minimizes cross-contamination and sample transposition events (Figure 5).

Quick instrument setup is enabled by use of prefilled, foil-sealed cartridges designed to fit the cartridge rack in only one orientation, PrepFiler sample and elution tubes, and AutoMate *Express* tips that come seated in their AutoMate *Express* tip holders for minimal manipulation. The instrument display walks through the setup process, which includes loading the cartridges into the cartridge rack, inserting the cartridge rack on the instrument, and loading the sample tubes, elution tubes, and tips in the tip holders and putting them into the tip and tube rack.



Figure 4. Closed system that minimizes the risk of contamination and transposition errors.

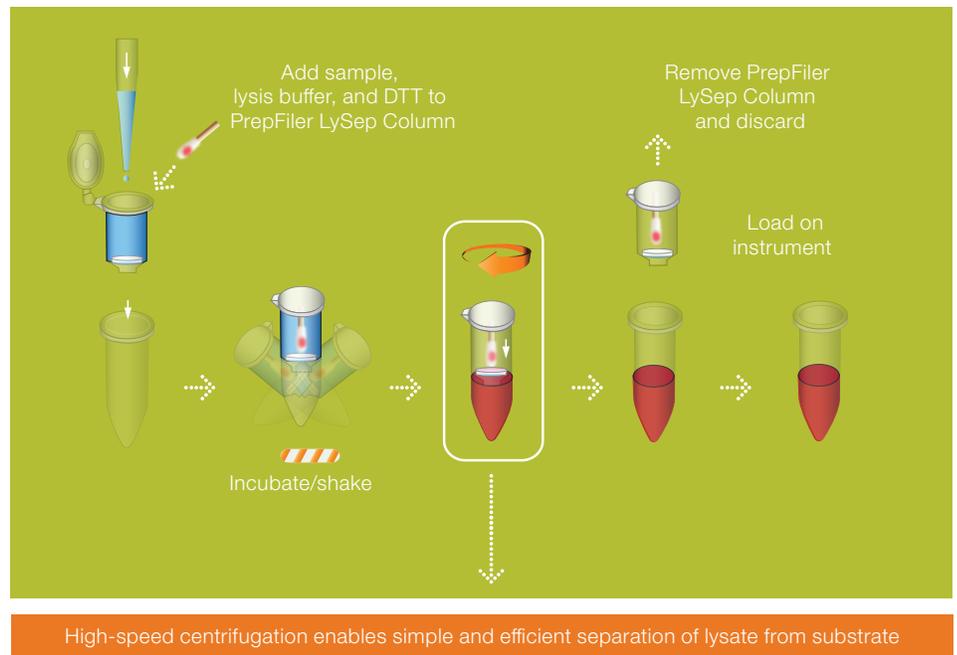


Figure 5. Improved method allowing users to easily perform lysis and effective substrate removal in a single-tube assembly.

Improved downstream performance of challenging samples

PrepFiler *Express* and PrepFiler *Express* BTA kits are part of the Applied Biosystems comprehensive portfolio of products for forensic laboratories. DNA extracted with PrepFiler *Express* and PrepFiler *Express* BTA kits on the AutoMate *Express* instrument has been tested and optimized to work with our latest quantification kits (Quantifiler Trio and Quantifiler HP kits) and STR kits (GlobalFiler kit, Applied Biosystems™ NGM Detect™, and Applied Biosystems™ Yfiler™ Plus PCR Amplification Kits) to maximize downstream performance (Figure 6).

The PrepFiler *Express* and PrepFiler *Express* BTA kits use a specially formulated wash solution developed to maximize the removal of common PCR inhibitors found in forensic samples while minimizing any loss of DNA during the purification steps. This enables clean, balanced DNA profiles to be generated that are easy to interpret.

The PrepFiler *Express* BTA kits were designed specifically for challenging samples such as bones, teeth, and adhesive base samples, including cigarette butts and tape lifts. Bone samples processed using the PrepFiler *Express* BTA kit on the AutoMate *Express* system yielded (in most cases) high-quantity and high-quality DNA to help generate interpretable STR profiles, with less number of steps compared to the traditional, manual, lab-developed methods (Figure 7).

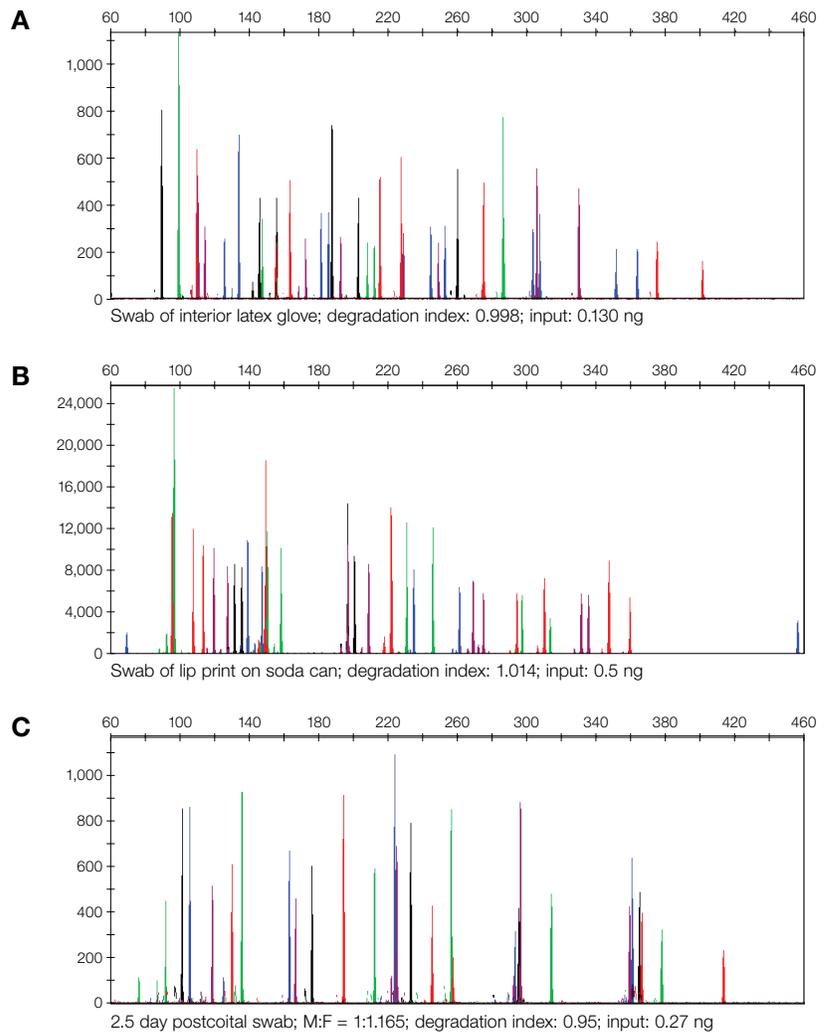


Figure 6. Representative electropherograms of forensic samples extracted with the PrepFiler *Express* kit on the AutoMate *Express* system and amplified using the (A) GlobalFiler, (B) NGM Detect, and (C) Yfiler Plus kits. All quantifications were performed using the Quantifiler Trio kit and run on the Applied Biosystems™ 3500 Genetic Analyzer.

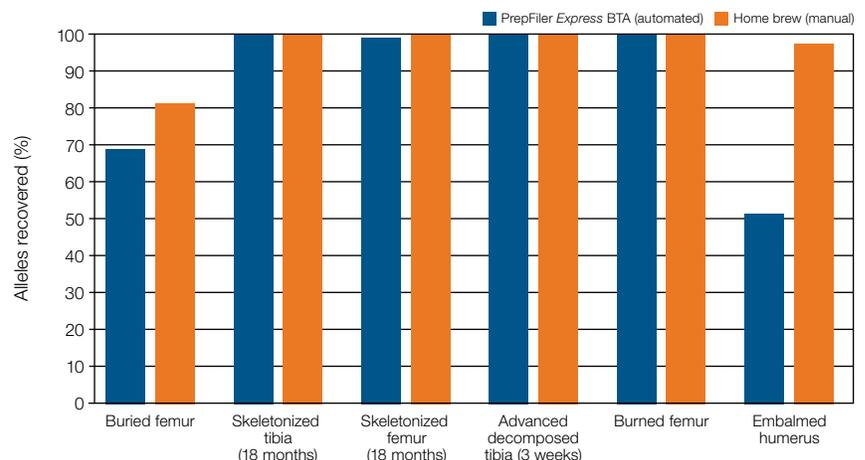


Figure 7. Generation of interpretable STR profiles from samples extracted with the PrepFiler *Express* BTA kit. DNA was extracted from bone samples of different levels of preservation and age with the PrepFiler *Express* BTA kit on the AutoMate *Express* system and quantified with the Quantifiler Trio kit. The extracts were then amplified with the GlobalFiler kit, run on the 3500 Genetic Analyzer, and analyzed. The percentage of alleles recovered was calculated. The PrepFiler *Express* BTA kit (blue bars) on the AutoMate *Express* system yielded comparable peak height (not shown) and number of alleles recovered to a manual lab-developed method (orange bars).

Ordering information

Description	Cat. No
AutoMate <i>Express</i> Forensic DNA Extraction System	4441763
AutoMate <i>Express</i> Forensic DNA Extraction System, with Service Install	4456582
AutoMate <i>Express</i> IC Protocol Card with Variable Elution (v1.1)	A33682
PrepFiler <i>Express</i> /AutoMate <i>Express</i> Validation	TRN00151
PrepFiler <i>Express</i> /AutoMate <i>Express</i> Performance Check	HPS10303
PrepFiler <i>Express</i> Forensic DNA Extraction Kit (52 cartridges)	4441352
PrepFiler <i>Express</i> BTA Forensic DNA Extraction Kit (52 cartridges)	4441351

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