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TECHNICAL NOTE

CRIMINALISTICS

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AutoMate Express™ Forensic DNA Extraction System for the Extraction of Genomic DNA from Biological Samples*

ABSTRACT: The AutoMate Express™ Forensic DNA Extraction System was developed for automatic isolation of DNA from a variety of forensic biological samples. The performance of the system was investigated using a wide range of biological samples. Depending on the sample type, either PrepFiler™ lysis buffer or PrepFiler BTA™ lysis buffer was used to lyse the samples. After lysis and removal of the substrate using LySep™ column, the lysate in the sample tubes were loaded onto AutoMate Express™ instrument and DNA was extracted using one of the two instrument extraction protocols. Our study showed that DNA was recovered from as little as 0.025 μL of blood. DNA extracted from casework-type samples was free of detectable PCR inhibitors and the short tandem repeat profiles were complete, conclusive, and devoid of any PCR artifacts. The system also showed consistent performance from day-to-day operation.

KEYWORDS: forensic science, DNA extraction, DNA purification, DNA isolation, DNA typing, automation, bench-top system

As the initial reports on DNA fingerprinting (1,2) more than two decades ago, DNA genotyping technology has undergone a remarkably rapid evolution (3–5). Short tandem repeat (STR) analysis has become an indispensable tool in forensic investigation. Forensic evidence poses unique challenges to the DNA extraction technologies undertaken as the first step in the STR profiling process. One such challenge is the broad and sometimes unpredictable nature of evidence sample types. Besides the inherent PCR inhibitors found in biological samples, PCR inhibitors may also arise from the substrate on which the biological samples are deposited and the environment in which the samples are exposed to. The forensic DNA extraction technology should be effective at removing a broad range of PCR inhibitors and have high DNA recovery efficiency so that successful STR profiles can be obtained. To improve forensic laboratories' ability to handle the ever growing number of forensic samples, the desirable DNA extraction technology should also be automated, easy to use, require minimal hands-on operation and be able to process multiple forensic samples simultaneously. Automation of DNA extraction also provides the benefits of minimizing contamination and producing more consistent DNA recovery.

A wide variety of methods based on different principles are available for the extraction of DNA for forensic applications. The phenol/chloroform extraction approach (6) is a sensitive method for the recovery of DNA from a wide variety of forensic samples. However, this procedure requires the use of large volumes of toxic phenol and chloroform solutions and is also tedious and time-consuming to perform. Chelex® 100 resin (7) (Bio-Rad Laboratories, Hercules, CA) extraction methods are popular in the forensic science community because they save time, reduce costs, simplify extractions, reduce safety risks, and minimize potential for contamination. However, because the Chelex® procedure does not include a purification step, inhibitors may not be effectively removed. Further, DNA gets degraded as a result of exposure to high temperatures during the extraction process. Therefore, if samples contain inhibitors or contaminants by increasing the sample size, one also increases the concentration of these substances in the DNA extract that can inhibit PCR. There are also concerns of potential degradation for long-term storage of isolated DNA samples.

Bench-top automated systems that enable isolation of DNA from sample lysate have gained importance in recent years. Biorobot EZ1 (Qiagen, Valencia, CA), Maxwell® 16 (Promega, Madison, WI), and iPrep (Invitrogen, Carlsbad, CA) are being used in forensic laboratories. These systems provide the advantages of low throughput, hands-free operation, and clean processing of evidence samples. It is important to note that the chemistry for extraction of DNA plays a key role in the quality and quantity of DNA obtained. Bench-top systems are a means of automating the liquid handling steps for various DNA extraction methods. The PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA) enables the isolation of DNA from a variety of biological samples that contain small quantities of biological material in such a way that substances that interfere with PCR are removed. Additionally, the DNA extract is of sufficiently high concentration that the

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volume of extract used for downstream analysis is minimal (8). The Prepfiler™ Kit is based on a unique chemistry wherein DNA from the lysate binds to the magnetic particles by formation of a complex that remains stable during the washing steps for effective removal of inhibitors and is ultimately dissociated in the elution buffer conditions. The PrepFiler™ Kit was designed specifically to support both manual and automated extraction of DNA from forensic samples (8,9).

The AutoMate Express™ Forensic DNA Extraction System (Applied Biosystems) described here is developed for automated extraction of DNA from forensic-type samples. The Instrument uses Patented Magtration® (magnetic filtration) technology, which traps magnetic particles against the sidewall of the pipetting tip. A simple bind-wash-elute procedure eliminates filtration and centrifugation steps from the DNA isolation process. The tip and tube rack is designed for stable mounting of elution tubes. All liquid handling operations are performed away from the sample and elution tubes to reduce the risk of contamination. By automating the PrepFiler™ chemistry on the AutoMate Express™, the system enables consistent recovery of DNA in high yields from forensic samples while effectively removing the most commonly encountered potent inhibitors of PCR. In addition, we have developed the PrepFiler Express BTA™ kit for the extraction of DNA from bones, teeth, and adhesive containing substrates. In this article, we report on the validation studies performed during the development of the AutoMate Express™ Forensic DNA Extraction System for the extraction of genomic DNA from biological samples.

Materials and Methods

Biological samples, such as blood and semen, were obtained from the Serological Research Institute (Richmond, CA). The Eppendorf Thermomixers were purchased from VWR Scientific Products (Batavia, IL). Indigo, hematin, and humic acid were obtained from Sigma-Aldrich (St. Louis, MO). Urban dust is a standard reference material (SRM 1649a) obtained from the National Institute for Standards and Technology (Gaithersburg, MD). The Quantifiler® Duo DNA Quantification Kit, AmpFℓSTR ® Identifiler®, Identifiler® Plus and MiniFiler™ PCR Amplification Kits, 7500 Real-Time PCR System, 3130xl Genetic Analyzer and associated software were from Applied Biosystems. All other chemicals used in this study were of analytical grade.

The Operation of LySep™ Column Assembly for Sample Lysis

A typical forensic DNA extraction workflow consists of lysing biological sample, separating lysate from substrate and purifying DNA from lysate. One of the common practices for separating lysate from substrate involves the use of a spin basket device. However, the manual transfer of lysate and substrate into spin basket is not only tedious; it could also result in spilling, sample loss, and contamination. To overcome these issues, we developed PrepFiler LySep™ column, an apparatus which allows sample lysis and substrate separation to occur in a single tube. Before use, the LySep™ column (Fig. 1*a*) was inserted inside a sample tube (Fig. 1*b*) to make a LySep™ column assembly (Fig. 1*c*). Sample and lysis buffer were then added sequentially to the LySep™ column assembly. The lysis reaction was carried out on a thermal mixer. The frit and membrane mounted at the bottom of the LySep™ column held the lysis buffer and sample in the top portion. After lysis, the LySep™ column assembly was centrifuged. The membrane at the bottom of the LySep™ column deformed during centrifugation and allowed the lysate to pass into the sample tube while the frit trapped the substrate in the LySep™ column. The LySep™ column and substrate was then removed and discarded and the sample tube containing lysate was now ready for DNA extraction on the AutoMate Express™ instrument.

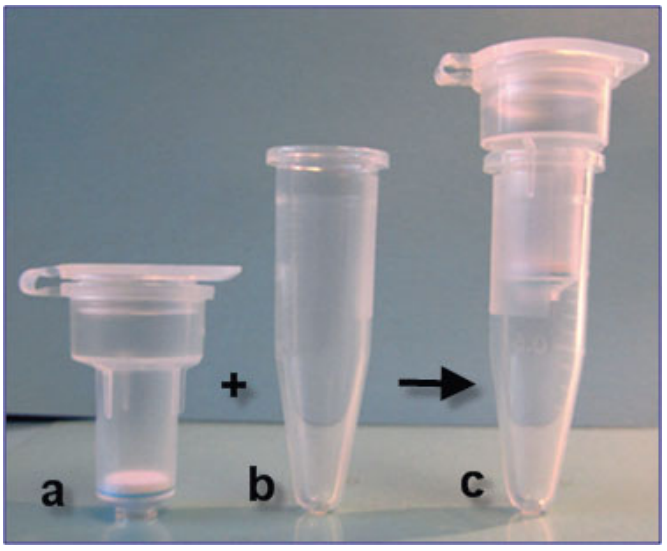

FIG. 1—*(a) LySep™ column; (b) sample tube; (c) LySep™ column assembly.*

Extraction of DNA

The DNA from anonymous donor samples (blood, saliva, and semen, either liquid or stains, and buccal swabs) was extracted using the PrepFiler Express™ Forensic DNA Extraction Kit. The DNA from bone, tooth, chewing gum, and cigarette butt samples was extracted using the PrepFiler Express BTA™ Forensic DNA Extraction Kit. Cell lysis using the PrepFiler Express™ Kit was performed by adding 500 μL of the PrepFiler lysis solution to the biological sample in a LySep™ column assembly. The PrepFiler lysis solution is prepared by mixing 500 μL of PrepFiler lysis buffer and 5 μL of 1.0 M freshly prepared dithiothreitol (DTT). The lysis mixture was incubated at 70°C for 40 min with shaking at about 750 rpm using an Eppendorf Thermomixer. Following lysis, the LySep™ column assembly was centrifuged for 2 min at $10{,}000 \times g$ to transfer the lysate to the sample tube. The lysate in the sample tube was processed on the Automate Express Forensic DNA extraction instrument using the PrepFiler Express™ instrument protocol.

Cell lysis for bone and tooth samples was performed in the PrepFiler™ Bone and Tooth Lysate Tube by adding 220 μL PrepFiler BTA™ Lysis Buffer, 3 μL freshly prepared 1.0 M DTT and 7 μL Proteinase K (20 mg/mL) to 50 mg bone or 5 mg of tooth powder (unless specified). The lysis mixture was incubated at 56°C for 2 h with shaking at about 1100 rpm using the Eppendorf Thermomixer. After lysis, the tube containing the bone or tooth lysate was centrifuged for 2 min at $10{,}000 \times g$, and then the supernatant was transferred to a PrepFiler™ Sample Tube. Cell lysis for chewing gum and cigarette butt samples was performed in a LySep™ column/sample tube assembly by adding 220 μL PrepFiler BTA™ Lysis Buffer, 3 μL freshly prepared 1.0 M DTT and 7 μL Proteinase K (20 mg/mL). The lysis mixture was incubated at 56°C for 40 min with shaking at about 750 rpm using the Eppendorf Thermomixer. After lysis, the LySep™ column assembly was centrifuged for 2 min at $10{,}000 \times g$ to transfer the lysate to the sample

tube. The lysate generated using PrepFiler BTA™ Lysis Buffer was purified on the Automate Express Forensic DNA extraction instrument using the PrepFiler Express BTA instrument protocol.

The DNA from the biological samples was also extracted, whenever mentioned, using the DNA IQ® Casework Sample Kit for Maxwell® 16 and the EZ1 DNA Investigator Kit. The procedure recommended by the manufacturers for the respective extraction kit was followed. The elution volume in all cases was 50 μL.

The DNA extracts obtained were stored at 4°C and −20°C for short- and long-term storage, respectively.

Quantitation of DNA

Extracted DNA was quantified using the Quantifiler® Duo DNA Quantification Kit (10) on an Applied Biosystems 7500 Real-Time PCR System according to manufacturer recommended procedures. Samples of the same type were grouped together in the same qPCR plate to avoid introducing run-to-run variation. The quantitation results were analyzed using SDS Software v1.2.3 (Applied Biosystems).

STR Analysis

Quantified DNA was processed for STR profiling using the AmpFℓSTR® Identifiler® PCR Amplification Kit (11) according to manufacturer recommended procedures. Some samples were also processed for STR profiling using the Identifiler® Plus (12) or Minifiler™ (13) Kits following the protocols recommended by the manufacturer and are described in respective studies. A total of 1 ng of human DNA or up to a maximum of 10 μL of extracted DNA was used for STR PCR amplification. Samples were amplified on a GeneAmp® 9700 thermal cycler, electrophoresed on 3130xl Genetic Analyzers, and analyzed using GeneMapper® *ID-X* Software v1.0 according to manufacturer recommended procedures (Applied Biosystems).

Extraction of Blanks

Extraction blanks were processed following the protocol used for biological samples. They were extracted as described earlier and two aliquots of 2 μL each from the 50 μL eluate were used for quantification. A 10 μL aliquot of the eluate was used for STR analysis as described earlier.

Sensitivity Studies

DNA from 0.025, 0.1, 0.25, 1.0, and 5.0 μL of liquid blood was extracted using the PrepFiler Express™ Kit. For the liquid blood samples with input amount of <1 μL, the liquid blood was first diluted in 1× PBS in such way that 5 μL of diluted blood was equivalent to the required amount. All samples were extracted in four replicates along with one extraction reagent blank (XB) included for each instrument run. The DNA was quantified and processed for STR analysis as described earlier.

Reproducibility Studies

Five microliter epithelial cell suspension on swab, 1 μL semen on cotton cloth, and 2 μL bloodstain on cotton cloth samples were processed using the PrepFiler Express™ Kit. Fifty milligram pulverized bone powder and 5 mg pulverized tooth powder were processed using the PrepFiler Express BTA™ Kit. Four replicate DNA extractions were performed on each of the five sample types with one extraction blank included in each extraction run. Each sample set was extracted three times (one per day on three different days). The DNA was quantified and processed for STR analysis as described earlier.

Stability Studies

One microliter blood on blue denim (Canyon River) and 1 μL blood on cotton with inhibitor mix (12.5 mM indigo, 0.5 mM hematin, 2.5 mg/mL humic acid, and 300 mg/mL urban dust) were processed using the PrepFiler Express™ Kit. Ten milligram pulverized tooth powder (aged approximately 6 months, exposed to environment/light for 3 days) and 50 mg pulverized bone powder were processed using the PrepFiler Express BTA™ Kit. Three replicate DNA extractions were performed on each of the four sample types along with one extraction blank included in each extraction run. Each sample set was extracted once, then quantified and processed for STR analysis as described earlier.

Case-Type Samples

DNA from the following samples was extracted with the PrepFiler Express™ Kit in triplicate: (i) 2 μL dried blood on acetate fabric, (ii) 1 μL blood on 5-mm black leather punch, (iii) 2 μL blood on rayon fabric, (iv) 2 μL blood on silk fabric, (v) 2 μL blood on wool fabric, (vi) 3 μL diluted blood (1:10) on cotton cloth, (vii) hair root, (viii) blood mixture: 1 μL from men and 9 μL from women, (ix) 50 μL saliva stain on cotton, (x) 5 μL saliva on 5-mm FTA® punch (Whatman, Inc., Clifton, NJ), (xi) epithelial cell fractions of a mock sexual assault sample, and (xii) sperm fractions of a mock sexual assault sample. The mock sexual assault samples were prepared by mixing 2 μL sperm positive semen with 50 μL vaginal epithelial cell suspension. Epithelial cell fractions (DE-e fraction) and sperm fractions (DE-s fraction) were generated from the mock sexual assault samples using the procedure described by Gill et al. (14). Following lysis, 50 μL of DE-e fraction was added to 450 μL of PrepFiler™ Lysis Buffer, then processed for extraction using the PrepFiler Express™ protocol on the AutoMate Express™ instrument. The DE-s fraction (sperm pellet) was processed according to the PrepFiler Express™ Forensic DNA Extraction Kit for semen samples. DNA from the following samples was extracted using the PrepFiler Express BTA™ Kit in triplicate: (13) Airwave gum (chewed for 30 min, 1/8 of one piece) and (14) Marlboro Light cigarette butt (approximately ¾ cm cutting of filter paper). One extraction blank was included in each extraction run. Each sample set was extracted once, then quantified and processed for STR analysis as described earlier.

Contamination Study

Two independent contamination studies were conducted more than 12 months apart on the same AutoMate Express™ instrument. Extraction blanks and blood samples were arranged in an alternating pattern in the AutoMate Express™ tip and tube rack in each run in both contamination studies. In the first contamination study, there were twenty 10 μL blood samples and 19 extraction blanks. In the second contamination studies, there were 65 blood samples and 65 extraction blanks; the input volume of the blood samples were 20, 25, 30, 35, and 40 μL (13 samples for each blood input volume). All 84 extraction blanks from the two sets of contamination studies were processed for STR profiling with the Identifiler® Kit. Any extraction blank that exhibited peaks above 50 RFU were

additionally processed for STR profiling in triplicate with the MiniFiler™ Kit.

Correlation Studies

DNA from the following samples was extracted using the AutoMate Express™, Maxwell® 16, and EZ1® Advanced XL instruments and corresponding reagent kits from each instrument platform: (i) 0.3 μL blood on cotton cloth, (ii) 2 μL blood on 5-mm cotton cloth, (iii) 2 μL blood on FTA® paper, (iv) 50 μL epithelial cell suspension on swab, (v) 1 μL semen on 5-mm cotton cloth, (vi) 1 μL bloodstain on 5-mm blue denim, and (vii) 50-mg pulverized bone powder. With the AutoMate Express™ System, bone samples were processed using the PrepFiler Express BTA™ Kit. All other sample types were processed with the PrepFiler Express™ Kit. With the EZ1® Advanced XL instrument, samples were prepared and extracted using reagents in the EZ1® DNA Investigator Kit from Qiagen according to the manufacturer's recommendations (15). Bone samples were incubated with 400 μL of 0.5 M EDTA at 37°C for 40 h, then for 3 h with proteinase K. One microliter of carrier RNA solution (1 μg) was added to each lysate prior to DNA extraction on the instrument. With the Maxwell® 16 instrument, samples were prepared and extracted using reagents in the DNA IQ™ casework sample kit from Promega according to the manufacturer's recommendations (16,17), with the exception that the bone protocol was modified to accommodate a 50 mg input of bone powder sample. The following protocol was used to prepare bone samples for DNA extraction on the instrument: (i) Prepare a proteinase K digestion solution by mixing 9.44 mL of bone incubation buffer with 560 μL of stock proteinase K solution. (ii) Add 500 μL of freshly prepared proteinase k digestion solution to the sample. (iii) Incubate the sample tube at 56°C for 1 h. (iv) Centrifuge the sample tube at 10,000 × *g* for 5 min to separate the remaining bone substrate. (v) Transfer the solution to a new 1.5-mL tube. (vi) Add 400 μL of lysis buffer to the solution, then vortex briefly.

For each method, DNA extractions were performed on six replicates of the bone sample and 12 replicates of each of the first six sample types. One extraction blank was included in each extraction run. Extracted DNA was set up for qPCR prepared on the HID EVOlution™—qPCR/STR Setup System using the Quantifiler® Duo DNA Quantitation Kit (18). Each extracted sample was quantified once. After quantitation, samples were normalized and set up for amplification using the HID EVOlution™—qPCR/PCR Setup System with the Identifiler® Kit (18).

Results and Discussion

The AutoMate Express™ Forensic DNA Extraction System (AutoMate Express™ System) is designed specifically for the extraction of DNA from forensic samples. The system consists of the PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits and the AutoMate Express™ instrument. The PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits contain disposable reagent cartridges and other necessary reagents and plasticware for lysis of cells, generation of a clear lysate and hands-free purification of DNA from the lysate. The developmental validation studies were performed following the revised validation guidelines provided by the Scientific Working Group on DNA Analysis Methods Guidelines (19). These guidelines describe the quality assurance requirements that a laboratory should follow to ensure the quality and integrity of the data and the competency of the laboratory. The experiments focus on kit performance parameters relevant to the intended use of the kits as the extraction of genomic DNA is a part of the forensic DNA genotyping procedure. By testing the procedure with samples commonly encountered in forensic and parentage laboratories, the validation process clarifies attributes and limitations that are critical for sound data interpretation in casework.

Sensitivity Studies

Sensitivity studies were performed to determine the range of biological sample amounts that can be reliably processed for the extraction of genomic DNA using the AutoMate Express™ System. Table 1 shows the average DNA yield for each replicate set. The DNA yield increased proportionately with increasing sample volumes. DNA was effectively recovered from the smallest sample amount tested (0.025 μL liquid blood). The efficiency of genomic DNA extraction remained linear up to the maximum volume of blood tested (5 μL) with R^2 value of 0.9996.

The ability to remove PCR inhibitors from a sample by the PrepFiler Express™ system was monitored by the C_T values for the internal PCR control (IPC) in the Quantifiler® Duo DNA quantification Kit (10). If the DNA extract contains PCR inhibitors, one would typically expect an upward shift of >1 IPC C_T value for the sample compared with the IPC C_T value for the nontemplate control (NTC). The average IPC C_T values for the blood samples of all five input amounts is 29.7 with a standard deviation of 0.09 and 29.4 for NTCs with a standard deviation of 0.08. The difference between the IPC C_T of a sample and that of NTCs is within ±1 C_T unit, indicating that PCR inhibitors were effectively removed during extraction. The IPC C_T values for the extraction blanks and NTCs were also within ±1 C_T unit, indicating that the PrepFiler Express™ Kit reagents did not introduce PCR inhibitors into the extracted DNA.

The quality of the DNA extract obtained from the AutoMate Express™ System was further evaluated by examining the STR profiles. Representative STR profiles are shown in Fig. 2. Full, conclusive STR profiles were obtained from all samples in the sensitivity study.

Reproducibility Studies

Studies were performed to assess the reproducibility of the quantity and quality (as judged by the presence of PCR inhibitors) of DNA obtained from replicate extractions of biological samples. The average DNA yield for the reproducibility study samples are shown in Fig. 3. The insert in Fig. 3 is the enlarged view of the DNA yield for bone samples. Consistent DNA concentrations were obtained for each sample types except for tooth samples. The variation in concentrations from 5 mg of tooth powder samples is likely due to the sample amount variation originated either from nonuniformity of the sample or from measuring very small amount of tooth powder. Average IPC C_T values for the samples and NTCs

TABLE 1—*Sensitivity study: average DNA yield.*

Blood Sample Volume (μL)	Average DNA Yield, ng (n = 4)	SD
5	144.89	21.7
1	26.03	2.7
0.25 (5 μL of a 1:20 dilution)	6.39	1.1
0.1 (5 μL of a 1:50 dilution)	2.68	0.7
0.025 (5 μL of a 1:200 dilution)	0.65	0.3
Extraction blank	0	

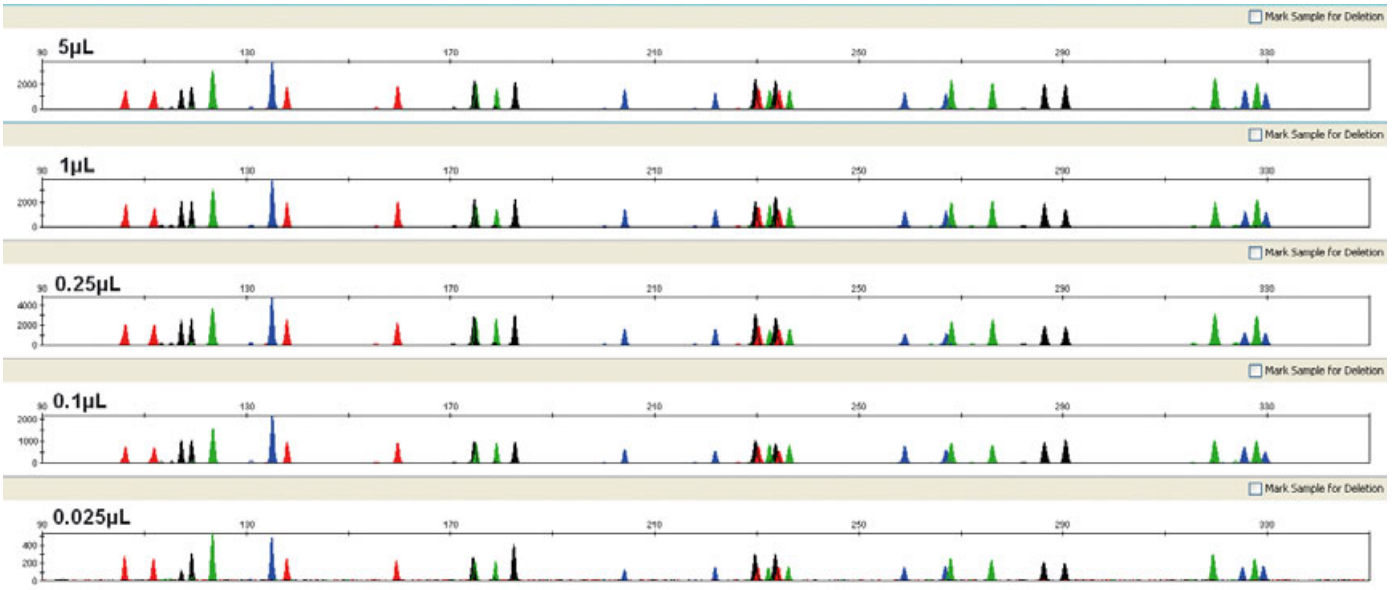

FIG. 2—*Sensitivity studies: representative Identifiler® short tandem repeat profiles of DNA extracted from different input amount of liquid blood.*

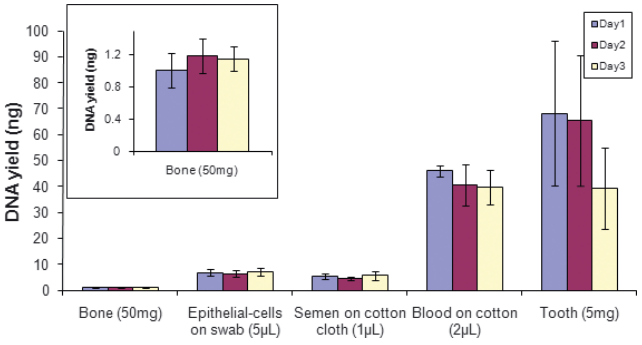

FIG. 3—*Reproducibility studies: average DNA yield from different mock forensic samples processed on three consecutive days. Four replicate DNA extractions were performed on each of the five sample types.*

TABLE 2—*Stability study: DNA concentration and total yield.*

Sample type	Average DNA Concentration, ng/µL (n = 3)	Average Total Yield, ng (n = 3)	SD
1 µL blood on blue denim	0.51	26.85	6.39
1 µL blood on cotton with inhibitor mix	0.46	23.76	4.92
10 mg tooth powder exposed to environment/light	0.16	8.36	7.04
50 mg aged bone powder	0.01	0.49	0.15
Extraction blank	0	0	

were within ±1 C_T unit, indicating that PCR inhibitors present in all tested sample types were effectively removed during the extraction of DNA using the AutoMate Express™ System.

The quality of the DNA extracts obtained from the AutoMate Express™ System was further evaluated by examining the STR profiles. Full and balanced STR profiles were obtained from all samples with the exception of the bone samples, which did not return full profiles owing to sample degradation (data not shown).

Stability Studies

Stability studies were performed to determine the ability of the AutoMate Express™ System to extract DNA and remove PCR inhibitors from samples subjected to environmental and chemical insults. A wide variety of compounds which may inhibit PCR have been reported, for example, hematin, humic acid, dyes, etc. The physicochemical properties of some of these compounds are similar to that of DNA and are co-extracted with the DNA. It is important to remove such PCR inhibitors during isolation of DNA. Further, it is critical that the extraction reagents themselves do not introduce PCR inhibitors in the sample. The sample types used in the stability study were specifically selected as each one puts forth different challenges. Blood samples spiked with an inhibitor mix tested the ability of the system to remove PCR inhibitors. The blood stain on denim contained inhibitory dyes that may be co-extracted with the DNA. Samples exposed to the environment may face other environmental insults such as UV light and heat.

The average DNA concentration and yield for the stability study samples are shown in Table 2. The variation in concentrations between sample types of 1 µL blood on blue denim and 1 µL blood on cotton with inhibitor mix is within the expected variation introduced through the extraction and quantitation procedures. Thus, the PrepFiler Express™ Kit is capable of efficiently extracting the genomic DNA from samples that are exposed to environmental and chemical insults. Average IPC C_T values for the samples and NTCs were within ±1 C_T unit, indicating that PCR inhibitors present in all tested sample types were effectively removed during the extraction of DNA using the AutoMate Express™ System.

The quality of the DNA extract obtained from the AutoMate Express™ System was further evaluated by examining the STR profiles. Representative STR profiles are shown in Figs 4 and 5. Full and balanced STR profiles were obtained from blood on blue denim and blood on cotton with inhibitor mix samples (Fig. 4). A ski slope effect was observed for both tooth and bone samples

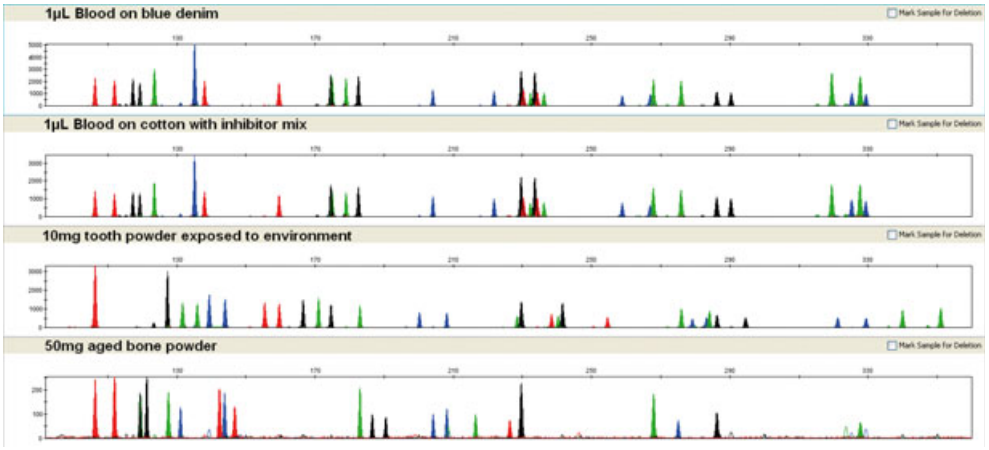

FIG. 4—*Stability studies: representative Identifiler® short tandem repeat profiles of challenging mock forensic samples.*

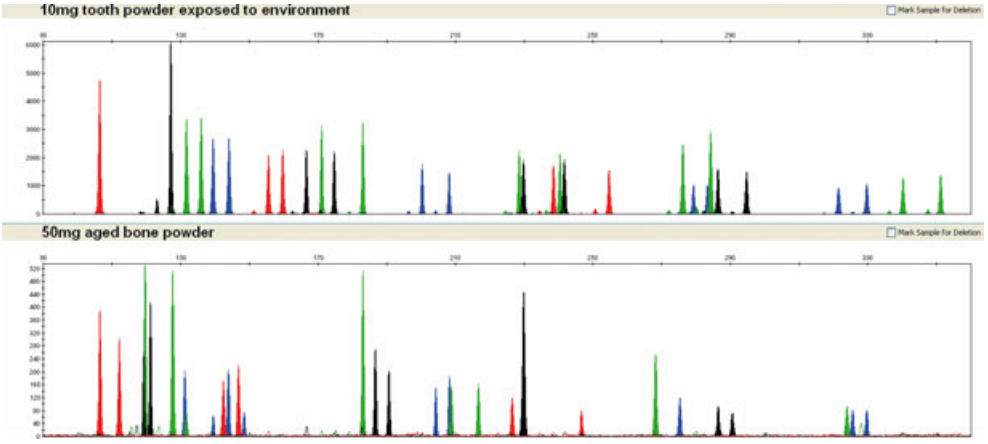

FIG. 5—*Identifiler® Plus short tandem repeat profiles from DNA extracted from tooth and bone sample in stability studies.*

(Fig. 4). Allelic drop-out was also observed for the larger loci of the bone samples. The ski slope effect and allelic drop-out were still observed after analyzing the bone and tooth samples with the Identifiler® Plus Kit, which provides a high level of tolerance for known PCR inhibitors introduced by forensic samples (12) (Fig. 5). Therefore, these effects are likely due to sample degradation.

Case-Type Samples

This experiment was performed to evaluate the extraction of genomic DNA from different sample types that are commonly processed in a forensic laboratory using the PrepFiler Express™ Kit. Forensic-type samples were prepared using different substrates and human biological fluids (saliva, blood, and semen) as described in Materials and Methods. The average DNA yield for case-type samples is shown in Fig. 6. Variation in DNA yield may occur due to cells that are entrapped and/or bound within the substrate and are inaccessible to the lysis buffer. Variation in DNA concentrations between samples was expected because of the variation in the amount of biological material present in different samples from different donors and different body fluids. All sample types provided DNA in sufficient quantities for downstream applications. The IPC C_T values for the samples and NTCs were within ±1 C_T unit, indicating that PCR inhibitors present in all tested sample types were effectively removed during the extraction of DNA using the AutoMate Express™ System. Conclusive STR profiles were obtained from all samples and profiles for subset of the samples are shown in Fig. 7.

Contamination Studies

Two contamination studies were performed more than 12 months apart to assess the capability of the AutoMate Express™ instrument liquid handling system against cross-contamination. In the first contamination study, twenty 10-µL blood samples and 19 extraction blanks were arranged in three sets of AutoMate Express™ tip and tube racks in an alternating pattern and processed for extraction. The extract from extraction blanks were analyzed using AmpF*l*STR® Identifiler® PCR Amplification Kit. One of the 19 extraction blank samples exhibited a single peak with a height of 70 RFU at the vWA locus (data not shown). The sample was then processed for STR profiling in triplicate with the highly sensitive MiniFiler™ Kit. The vWA peak could not be reproduced, because of the absence of this locus in the MiniFiler Kit. However, the STR profiles generated with the MiniFiler Kit did not exhibit any alleles, so the peak was determined to be spurious. In the second contamination study, which was conducted a year later, total 65 extraction blanks and 65 blood samples were processed on 10

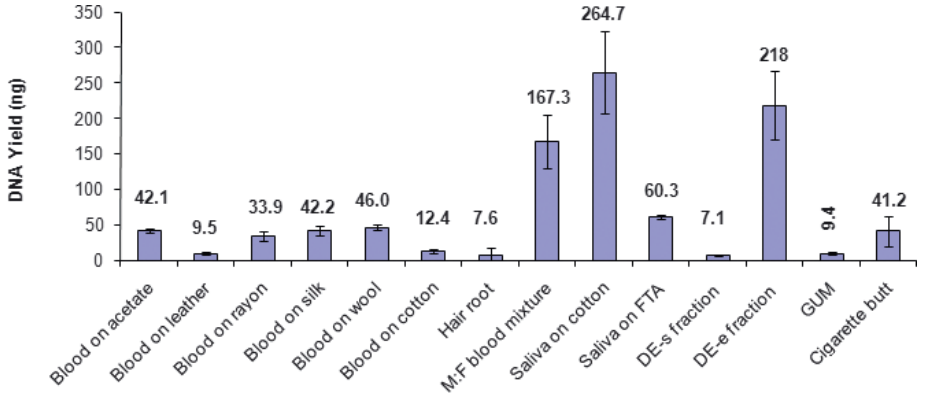

FIG. 6—*Case-type sample studies: average DNA yield from case-type samples. Three replicate DNA extractions were performed on each sample types.*

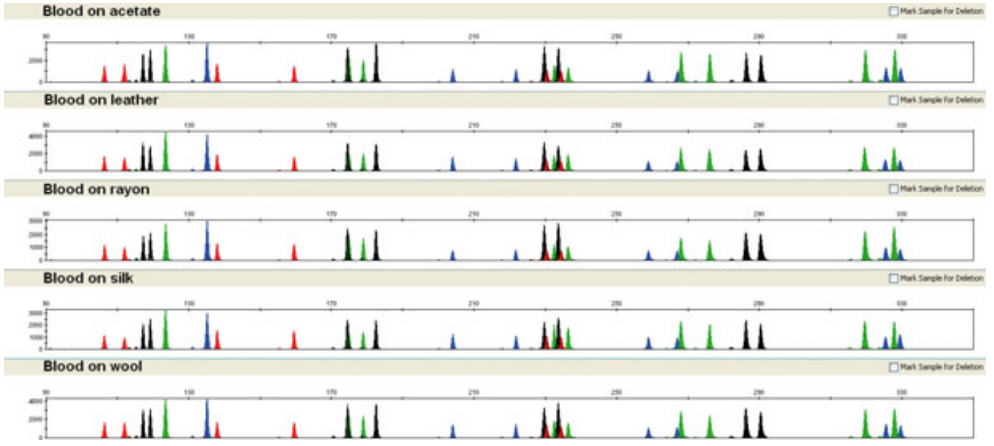

FIG. 7—*Case-type sample studies: representative Identifiler® short tandem repeat profiles from 2 μL dried blood on acetate fabric, 1 μL blood on 5-mm black leather punch, 2 μL blood on rayon fabric, 2 μL blood on silk fabric, and 2 μL blood on wool fabric.*

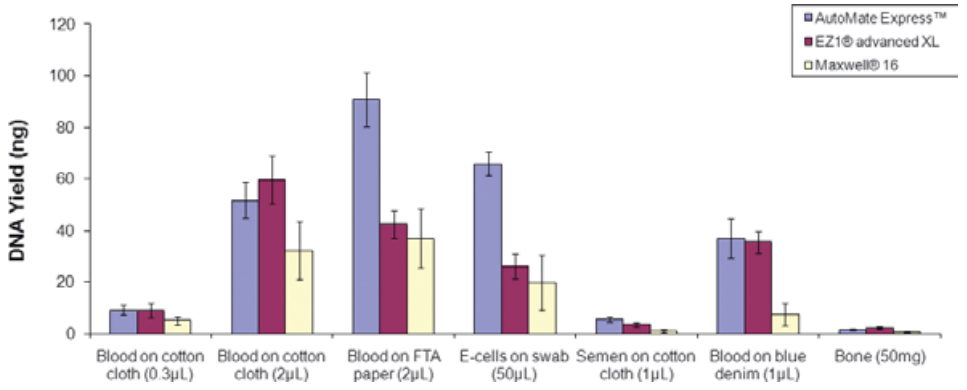

FIG. 8—*Correlation studies: average DNA yield from different mock forensic samples processed on three DNA extraction platforms. DNA extractions were performed on six replicates of the bone sample and 12 replicates of each of the other six sample types.*

extraction runs with each extraction blank located next to a liquid blood sample. Blood input volumes were 20, 25, 30, 35, and 40 μL (13 samples for each input volume). After DNA extraction, the extracts from extraction blanks were analyzed using AmpF*l*STR® Identifiler® PCR Amplification Kit. None of the 65 extraction blank samples exhibited peaks above 50 RFU. The results from both contamination studies indicates that the liquid handling system of the AutoMate Express™ instrument did not introduce any detectable DNA contamination during its operation over time.

Correlation Studies

Correlation studies were performed to evaluate the quality (as judged by the presence of PCR inhibitors) and quantity of DNA obtained using the AutoMate Express™ System as compared to

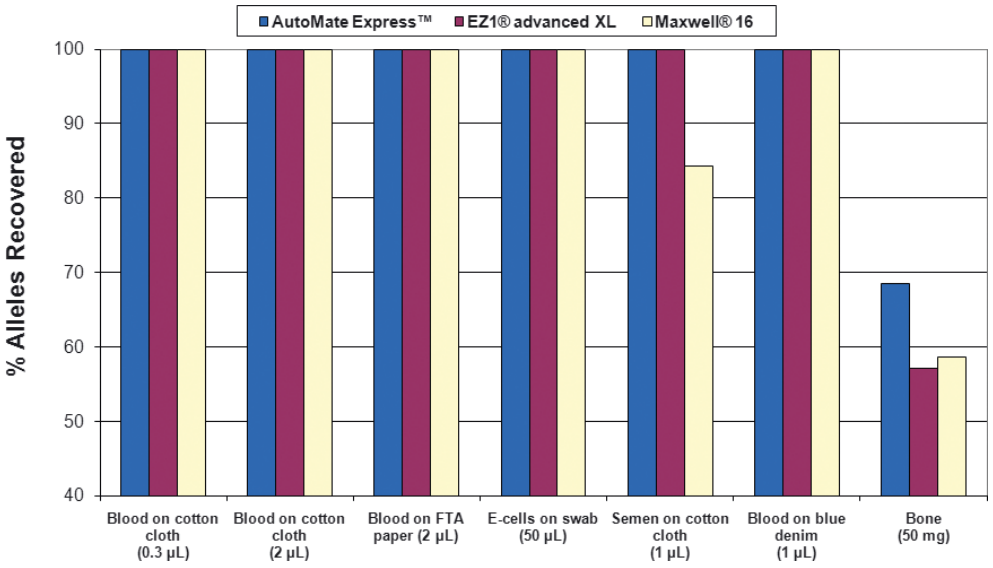

FIG. 9—*Correlation studies: the percentage of alleles recovered from mock forensic samples processed on three DNA extraction platforms.*

the DNA extract obtained using other commercially available methods.

The quantitation results for each extraction method were compared (Fig. 8). For all sample types investigated, the AutoMate Express™ System DNA yield and concentration was comparable with or higher than that of the other extraction methodologies. The IPC C_T values obtained using the AutoMate Express™ System and two other methods are all within 1 C_T of the extraction blank, indicating that PCR inhibitors present in all tested sample types were effectively removed. The AutoMate Express™ System obtained STR profiles with intracolor balance comparable with or better than the profiles obtained with Maxwell® 16 and EZ1® Advanced XL system and recovered more alleles for aged bone sample (Fig. 9).

Conclusions

The PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits and the AutoMate Express™ DNA Extraction instrument have been developed to extract genomic DNA from biological samples that are commonly observed in the forensic DNA laboratory. The system is capable of automatically processing 13 samples simultaneously. Validation studies demonstrate that the AutoMate Express™ System provides robust and reliable results in obtaining genomic DNA from forensic biological samples for downstream applications such as real-time quantitative PCR and PCR for STR profiling. The AutoMate Express™ System provides reliable results at different DNA input amounts and is effective in maximizing the amount of DNA obtained from samples that contain both small and large quantities of biological material. The utility of the extraction method in forensic DNA analysis was demonstrated using forensic-type samples. The DNA that was extracted was free of PCR inhibitors as determined by the IPC C_T values using the Quantifiler® Duo DNA Quantification Kit. The reagents and operations of the AutoMate Express™ System exhibited clean operations and did not introduce any detectable cross-contamination of human DNA.

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References

1. Jeffreys AJ, Wilson V, Thein SL. Hypervariable 'minisatellite' regions in human DNA. Nature 1985;314(6006):67–73.
2. Jeffreys AJ, Wilson V, Thein SL. Individual-specific 'fingerprints' of human DNA. Nature 1985;316(6023):76–9.
3. Edwards A, Civitello A, Hammond HA, Caskey CT. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 1991;49:746–56.
4. Roewer L, Epplen JT. Rapid and sensitive typing of forensic stains by PCR amplification of polymorphic simple repeat sequences in case work. Forensic Sci Int 1992;53:163–71.
5. Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM, Smerick JB, Budowle B. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. J Forensic Sci 2001;46:647–60.
6. Kochl S, Niederstatter H, Parson W. DNA extraction and quantitation of forensic samples using the phenol-chloroform method and real-time PCR. Methods Mol Biol 2005;297:13–30.
7. Walsh PS, Metzger DA, Higuchi R. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 1991;10:506–13.
8. Brevnov MG, Pawar HS, Mundt J, Calandro LM, Furtado MR, Shewale JG. Developmental validation of the PrepFiler Forensic DNA Extraction Kit for extraction of genomic DNA from biological samples. J Forensic Sci 2009;54:599–607.
9. Brevnov MG, Mundt J, Benfield J, Treat-Clemons L, Kalusche G, Meredith J, et al. Automated extraction of DNA from forensic sample types using the PrepFiler Automated Forensic DNA Extraction Kit. JALA 2009;14:294–302.
10. Barbisin M, Fang R, O'Shea CE, Calandro LM, Furtado MR, Shewale JG. Developmental validation of the Quantifiler Duo DNA Quantification kit for simultaneous quantification of total human and human male DNA and detection of PCR inhibitors in biological samples. J Forensic Sci 2009;54:305–19.
11. Applied Biosystems. AmpFℓSTR® Identifiler® PCR amplification kit user's manual, part # 4323291 Rev. B. Foster City, CA: Applied Biosystems, 2001.
12. Applied Biosystems. AmpFℓSTR® Identifiler® Plus PCR amplification kit user guide, part #: 4440211. Foster City, CA: Applied Biosystems, 2010.
13. Applied Biosystems. AmpFℓSTR® MiniFiler™ PCR amplification kit user guide, part #: 4374618. Foster City, CA: Applied Biosystems, 2007.
14. Gill P, Jeffreys AJ, Werrett DJ. Forensic application of DNA 'fingerprints.' Nature 1985;318(6046):577–9.
15. Qiagen. EZ1 DNA investigator handbook. Valencia, CA: Qiagen, 2009, http://www.qiagen.com/literature/default.aspx?Term=&Language=EN&

LiteratureType=1&ProductCategory=232&r = 1281 (accessed April 30, 2009).
16. Promega Corporation. DNA IQ™ casework sample kit for Maxwell® 16. Madison, WI: Promega Corporation, 2008.
17. Promega Corporation. Bone extraction protocol to be used with the DNA IQ™ system. Madison, WI: Promega Corporation, 2008.
18. Fang R, Liu JY, Kijenski HL, Benfield J, Wong A, Lagacé R, et al. The HID EVOlution system for automation of DNA quantification and short tandem repeat analysis. JALA 2010;15:65–73.
19. Scientific Working Group on DNA Analysis Methods (SWGDAM). Revised validation guidelines issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM). Forensic Sci Commun 2004;6(3):1–6. http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm#perfcheck (accessed November 24, 2010).

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