TECHNICAL NOTE

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Developmental Validation of the PrepFiler™ Forensic DNA Extraction Kit for Extraction of Genomic DNA from Biological Samples*

ABSTRACT: The PrepFiler™ Forensic DNA Extraction Kit enables isolation of genomic DNA from a variety of biological samples. The kit facilitates reversible binding of DNA with magnetic particles resulting in high DNA recovery from samples with very low and high quantities of biological materials: 0.1 and 40 μL of human blood (donor 2) provided 14 and 2883 ng of DNA, respectively. Following the revised SWGDAM guidelines, performance of the developed method was investigated using different sample types including saliva on swabs, semen stains on cotton fabric, samples exposed to environment, samples with polymerase chain reaction (PCR) inhibitors, blood stains (on denim, cotton cloth, and FTA® paper), and touch evidence-type samples. DNA yields for all samples tested were equal or better than those obtained by both phenol–chloroform extraction and commercial kits tested. DNA obtained from these samples was free of detectable PCR inhibitors. Short tandem repeat profiles were complete, conclusive, and devoid of PCR artifacts.

KEYWORDS: forensic science, DNA extraction, DNA purification, DNA isolation, DNA typing, short tandem repeat profiling

Profiling for short tandem repeat (STR) loci is routinely performed in forensic, DNA database and paternity applications for human identification, and lineage studies (1,2). The genotyping protocol is comprised of extraction of DNA from the biological sample, quantification of the DNA, amplification of STR loci, separation of amplified products using gel or capillary electrophoresis, and analysis of the results. The past two decades have witnessed advancements in the development of new technologies for STR analysis. These advancements include development of real-time polymerase chain reaction (PCR) methods for quantification of human DNA (3–7), multiplex STR kits for profiling of autosomal and Y-STRs (8–14), capillary electrophoresis instruments (15,16), fluorescence imaging systems (17), and data analysis programs (2,12,18–21).

Forensic analysts come across a variety of biological samples including stains of blood, saliva or semen on different substrates, swabs of body surface, hair, bones, and finger nail scrapings that are exposed to a range of environmental insults. DNA in the cells is associated with a number of physiological components and other macromolecules that protect the DNA *in vivo*. If these substances are not removed during the DNA extraction procedure, they can interfere in the downstream processes of DNA analysis such as PCR. Therefore, it is important that the procedure used for preparing the DNA is efficient and extracts the DNA in a highly purified form. A wide variety of methods based on different principles are available for extraction of DNA (2,22–26). These include Chelex® (Sigma-Aldrich, St. Louis, MO) extraction, phenol–chloroform, silica membranes, silica-coated magnetic beads, ion exchange membranes, and magnetic beads with an ionic surface. Kishore et al. (27) have proposed that silica-coated particles bind a certain portion of DNA in the sample *via* nonspecific adhesion providing low yields of DNA from samples containing smaller quantities of biological material. The principle of DNA extraction using phenol is mainly based upon denaturation of the contaminants including proteins and other macromolecules and isolation of DNA in purified form. It is important to note that the traditional phenol–chloroform organic extraction method, often referred to as gold standard, is still a predominant method for DNA isolation from forensic casework samples.

Isolation of DNA from forensic evidence samples, therefore, can be challenging and create bottlenecks in the sample processing workflow. The quality of DNA extract is of utmost importance as the ultimate goal of DNA analysis is to obtain an STR profile devoid of any PCR artifacts. In general, it is desirable for a forensic analyst to have an extraction methodology that enables: (i) the isolation of DNA from biological samples that contain small quantities of biological material; (ii) obtaining the DNA at a high concentration so that the volume of extract used for PCR is minimal; (iii) the removal of PCR inhibitors or substances that interfere with the PCR; (iv) the extraction of DNA from a variety of biological samples; and (v) the adaptation of the manual protocol and its chemistry to automation. We describe an innovative method, the PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems, Foster City, CA), that meets all of these criteria. The developed method enables the isolation of genomic DNA from forensic biological samples that is free of PCR inhibitors and ready for downstream applications such as real-time PCR and genotyping.

Materials and Methods

Biological samples such as blood, saliva, and semen were obtained from Serological Research Institute (Richmond, CA). Eppendorf Thermomixer R was from VWR Scientific Products

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*Part of the work was presented at the 60th Annual Scientific Meeting of the American Academy of Forensic Sciences, February 18–23, 2008, in Washington, DC. Some of the data is also presented in the PrepFiler™ Forensic DNA Extraction Kit User Guide.

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(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). Magnetic stand, Quantifiler® Human DNA Quantification Kit, AmpF/STR® Identifiler® PCR Amplification Kit, 7500 Real-Time PCR System, 3100 Genetic Analyzer and associated software were from Applied Biosystems (Foster City, CA). All other chemicals used in this study were of analytical grade. The samples were prepared as described in each pertinent section below using blood samples from two individual donors, saliva from two donors, and semen from a single donor. Touched objects such as soda can, hat, cell phone, steering wheel, and shoe were from anonymous donors.

Extraction of DNA

The DNA from anonymous donor samples (liquid or stains of blood, saliva, and semen on cotton cloth, denim, or cotton swabs) was extracted by using the PrepFiler™ Kit. Cell lysis was performed by adding 300 μL of the lysis buffer and 3 μL of 1.0 M Dithiothreitol (DTT) to the biological sample in a 1.5-mL microcentrifuge tube. An aliquot of 5 μL of DTT was used for samples containing semen. The lysis mixture was incubated at 70°C for 40 min with shaking at about 900 rpm using Eppendorf Thermomixer R. The incubation for saliva and semen samples was 20 and 90 min, respectively. The tube was centrifuged for 2 min to collect the condensate from the lid and contents of the tube were transferred to a PrepFiler™ filter column (Applied Biosystems). The lysate was separated from the substrate by centrifuging the filter column for 2 min at 16,110×*g*. Genomic DNA in the lysate was bound to the magnetic particles by adding 15 μL of a uniform suspension of magnetic particles (prepared by pulse vortexing the magnetic particle suspension tube) and 180 μL of binding solution (isopropanol) to the lysate. The tube was closed and incubated at room temperature with shaking (1000 rpm) for 10 min. The tube was placed on the magnetic stand for 1 min to separate the magnetic particles. The supernatant was discarded by using a pipette without disturbing the pellet. The magnetic particles were then resuspended in 300 μL of wash buffer, vortexed for 5 sec, placed on the magnetic stand, and the supernatant was removed and discarded. The wash step was repeated two more times. After three washes, the tube was kept on the magnetic stand with lid open for 7–10 min for evaporation of the residual organic solvents. To release the DNA from the magnetic particles, an aliquot of 50 μL of elution buffer was added and the tube was vortexed for 10 sec at maximum speed. It was then incubated at 70°C and 900 rpm for 5 min. Finally, the tube was placed on a magnetic stand for 1 min and the eluate was collected without disturbing the magnetic particles. The DNA extract was stored at 4°C and −20°C for short- and long-term storage, respectively.

The DNA from the biological samples was also extracted, where mentioned, using the standard phenol–chloroform method (26), DNA IQ™ System-Small Sample Casework Protocol (Promega Corporation, Madison, WI; Technical Bulletin TB296), EZ1 Investigator Kit (QIAGEN, Valencia, CA), and QIAamp® DNA Micro Kit (QIAGEN). The procedure recommended by the manufacturers for respective extraction kits was followed. The elution volume in all cases was 50 μL.

Quantification of DNA

The quantity of DNA was determined by the Quantifiler® Human DNA Quantification Kit (Applied Biosystems) using 2 μL of the DNA extract as described in the User's Manual (28). PCR was performed on the 7500 Real-Time PCR System and the data were analyzed using 7500 System SDS Software v1.2.3 (Applied Biosystems). Quantification of all sample eluates were run in duplicate except for the correlation studies where one replicate was run for each sample and all samples were quantified on a single plate in order to avoid experiment–experiment variation.

STR Analysis

The DNA extracts obtained from the biological samples using the PrepFiler™ Kit were amplified with the Identifiler® Kit using the procedure described in the User's Manual (29). The amplified products were analyzed on a 3100 Genetic Analyzer using GeneMapper® ID Software v3.2.1 as described in the User Guides (30,31).

Extraction of Blanks

Extraction blanks were processed exactly as regular samples. They were extracted as described above and two aliquots of 2 μL each from the 50 μL eluate were used for quantification. A 10-μL aliquot of the eluate was amplified using the Identifiler® Kit.

Sensitivity Studies

DNA from 0.1, 2.0, 5.0, 30.0, and 40.0 μL of blood samples from two donors was extracted using the PrepFiler™ Kit. All samples were extracted in triplicate. Extraction blanks in four replicates were included in the experimental set.

Reproducibility Study

DNA from the following samples was extracted in duplicate using the PrepFiler™ Kit (a 5-mm punch was used for extraction of all stains and a whole swab used in the case of swabs):

i) stains on noncolored cotton cloth prepared using 5 μL of human blood; samples were prepared using two donors (BSC-1 and BSC-2),
ii) swab samples prepared using 50 μL of human saliva; samples were prepared using two donors (SAL-1 and SAL-2), and
iii) semen stain on noncolored cotton cloth prepared using 1 μL of semen from a donor (SSC).

The extraction of DNA from the set of samples was performed on three different days. Each set of extraction contained extraction blanks in duplicate.

Stability Studies

DNA from the following samples was extracted in triplicate using the PrepFiler™ Kit (5-mm punch used for extraction in all cases):

i) stain on noncolored cotton cloth prepared using 5 μL of human blood spiked with 1 μL of inhibitor mix that contained indigo (12.5 mM), hematin (0.5 mM), humic acid (2.5 mg/mL), and urban dust extract (prepared by suspending 300 mg of the urban dust in 1 mL of 10 mM Tris, 0.1 mM EDTA, 8.0 pH; the suspension was shaken for 18 h at room temperature and 3 μL of supernatant was used for preparing 100 μL of inhibitor mix); the samples were prepared using two donors (BSCI-1 and BSCI-2),

ii) stain on denim prepared using 5 μL of human blood; the samples were prepared using two donors (BSD-1 and BSD-2), and
iii) blood stain on cotton cloth exposed to outdoor environment for 7 days; the samples were prepared using 10 μL of human blood from two donors (BSC7-1 and BSC7-2).

Extraction blanks in four replicates were included.

Case-Type Samples

DNA from the following samples was extracted in duplicate using the PrepFiler™ Kit (a 5-mm punch was used for extraction of all stains and a whole swab used in the case of swabs):

i) 2 μL of human liquid blood (LB),
ii) stain on FTA® paper (Whatman, Inc., Sanford, ME) prepared using 2 μL of human blood (BFTA),
iii) stain on noncolored cotton cloth prepared using 2 μL of human blood (BSC),
iv) stain on noncolored cotton cloth prepared using 2 μL of human blood spiked with inhibitor mix that contained indigo, hematin, humic acid, and urban dust extract (BSCI),
v) stain on denim prepared using 2 μL of human blood (BSD),
vi) stain on noncolored cotton cloth prepared using 1 μL of human semen (SSC),
vii) swab sample prepared using 50 μL of human saliva (SalSw),
viii) sperm and epithelial cell fractions (DE-s and DE-e fractions); the sexual assault-type swab samples were prepared by spiking 5 μL of sperm positive semen on female saliva swabs and were processed for differential lysis using the protocol described by Gill et al. (32). An aliquot of 150 μL of the epithelial cell fraction was mixed with 150 μL of PrepFiler™ lysis buffer and processed for isolation of DNA. Similarly, 200 μL of the sperm cell lysate fraction was mixed with 300 μL of PrepFiler™ lysis buffer followed by 15 μL of PrepFiler™ magnetic particles and 300 μL of isopropanol (binding solution). The binding mixture was then processed for isolation of DNA, and
ix) mixed stain on noncolored cotton cloth prepared using mixture of 1 and 4 μL blood from human male and female donors, respectively (MBSC).

DNA from the following touch evidence-type samples was extracted in single replicate:

x) swab of a soda can (SdcSw),
xi) swab of a hat (HSw),
xii) swab of a steering wheel (StwSw),
xiii) swab of a cell phone (CPhSw), and
xiv) swab of blood stain on shoe (BShSw).

Extraction blanks in duplicate were included.

Correlation Studies

DNA from the samples (i) through (vii) described in the Case-Type Sample Studies experiment was extracted in duplicate using the PrepFiler™ Kit, phenol–chloroform (26), DNA IQ™, the BioRobot® EZ1 Investigator Kit, and the QIAamp® DNA Micro Kit. Extraction blanks in duplicate were incorporated. The elution volume for all extraction methods was 50 μL.

Results and Discussion

The PrepFiler™ Kit is designed for the extraction of DNA from forensic samples, which is an integral step in the DNA analysis workflow. The kit contains reagents necessary for lysis of cells, binding of the DNA to the magnetic particles, removal of PCR inhibitors, and release of bound DNA. The developmental validation studies were performed following the revised validation guidelines provided by the Scientific Working Group on DNA Analysis Methods (SWGDAM) Guidelines (33). 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 the biological sample that can be reliably processed for the extraction of genomic DNA using the PrepFiler™ Kit. The results from different volumes of blood samples from the two donors tested are summarized in Fig. 1. Average concentration of DNA extracted ranged from 0.13 to 57.67 ng/μL for 0.1 μL–40 μL of blood, respectively. The magnetic particles in the PrepFiler™ Kit were effective in binding and releasing the genomic DNA from lysate of samples that contain low quantities of biological material (e.g., 0.1 μL of blood). Further, the magnetic particles were not saturated up to 40 μL of blood as demonstrated by the proportional increase in the yield of DNA as the volume of blood processed increases; total yield of DNA from 40 μL of blood was 2694 and 2883 ng for donors 1 and 2, respectively. The observed high dynamic range for processing of biological materials is attributed to the binding of DNA to the surface of the polymer-coated magnetic particles *via* physico-chemical interactions. The newly designed magnetic particles have a high capacity for DNA capture as the saturation of the particles was not observed up to 40 μL of LB samples investigated. Thus, even low and high amounts of biological material can be processed using the PrepFiler™ Kit. DNA yields may vary for

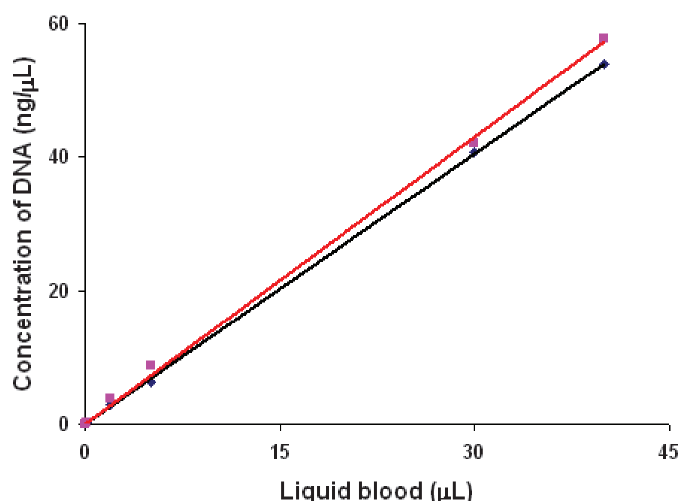

FIG. 1—*Sensitivity studies: average concentration of DNA in the extract obtained from 0.1, 2, 5, 30, and 40 μL blood samples from donor 1 (blue line) and donor 2 (red line).*

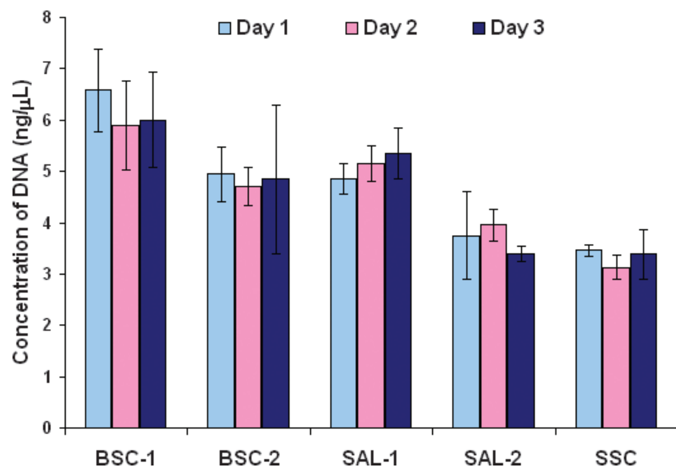

FIG. 2—*Reproducibility studies: average concentration of DNA in the extract obtained for different samples. BSC-1, blood (5 µL) stain on noncolored cotton cloth from donor 1; BSC-2, blood (5 µL) stain on noncolored cotton cloth from donor 2; SAL-1, saliva (50 µL) on swab from donor 1; SAL-2, saliva (50 µL) on swab from donor 2; SSC, semen (1 µL) stain on noncolored cotton cloth. The samples were extracted in duplicate and each extract was quantified in duplicate.*

different blood samples and are determined by the nucleated cell count of the blood sample.

Reproducibility Studies

A set of samples comprising blood stains, semen stains, and saliva on swab were extracted in duplicate using the PrepFiler™ Kit on three different days to assess the reproducibility of the extraction efficiency. Average DNA concentrations in the extract are presented in Fig. 2. The range, mean, and standard deviation values for the concentration of DNA are presented in Table 1. It is evident that the concentration of DNA in the extract for each sample was reproducible when the extractions were performed on three different days. As expected, the quantity of DNA varied, which is a combined result of the variations in the biological contents of the sample, and extraction and quantification procedures. The standard deviation values for the DNA yields from different samples studied ranged between 0.36 and 0.74.

TABLE 1—*Reproducibility studies: average concentration of DNA in the extracts.*

Sample*	Concentration of DNA (ng/µL)			
	Min	Max	Mean	SD
BSC-1	5.43	7.06	6.25	0.66
BSC-2	3.69	6.00	4.82	0.74
SAL-1	4.27	5.94	5.20	0.63
SAL-2	3.02	4.48	3.70	0.54
SSC	2.74	3.68	3.43	0.36

Min, max, and mean values were obtained from samples extracted over the course of 3 days (n = 6 total for each type).

*BSC-1, blood (5 µL) stain on noncolored cotton cloth from donor 1; BSC-2, blood (5 µL) stain on noncolored cotton cloth from donor 2; SAL-1, saliva (50 µL) on swab from donor 1; SAL-2, saliva (50 µL) on swab from donor 2; SSC, semen (1 µL) stain on noncolored cotton cloth. The samples were extracted in duplicate and each extract was quantified in duplicate.

SD, standard deviation.

Stability Studies

Stability studies were conducted to demonstrate the performance of the PrepFiler™ Kit when used to process samples subjected to environmental and chemical insults such as samples containing PCR inhibitors and exposed to environmental conditions. Forensic samples commonly contain compounds that inhibit the amplification of nucleic acids. These inhibitors, if not removed, can cause varying levels of reduced reaction efficiency, possibly complete inhibition of PCR. A wide variety of compounds which may inhibit PCR have been reported, e.g., hematin, humic acid, and dyes, etc. The physico-chemical properties of some of these compounds are similar to those of DNA and are co-extracted and purified with the DNA. It is important to remove such PCR inhibitors during isolation of the DNA. Further, it is critical that the extraction reagents themselves do not introduce PCR inhibitors in the sample. The samples used in the stability study were compromised and each one puts forth different challenges. Blood sample spiked with the inhibitor mix is a challenging sample in determining the ability of an extraction method to remove PCR inhibitors. Blood stain on denim is considered a challenging sample because inhibitory dyes are co-extracted from the denim. Samples exposed to the environment may get contaminated with soil and face other environmental insults.

The average concentration of DNA in the extracts from the samples investigated for the stability study is summarized in Table 2. The concentration of DNA for BSCI samples ranged from 4.53 to 5.50 ng/µL, for BSD from 2.88 to 4.24 ng/µL, and for BSC7 from 6.78 to 9.91 ng/µL. Thus, the PrepFiler™ Kit is capable of efficiently extracting the genomic DNA from samples that are exposed to the environmental and chemical insults. The DNA yields may vary for different blood samples, substrates, and environmental conditions.

The ability to remove PCR inhibitors from a sample using the PrepFiler™ Kit was monitored by the C_T values for the Internal PCR Control (IPC) in the Quantifiler® Human DNA Quantification Kit (3,28). If the DNA extract contains PCR inhibitors, one would typically expect an upward shift in the C_T value for the sample compared to the IPC C_T value for the nontemplate control (NTC). Figure 3 shows the average IPC C_T values for the stability studies samples obtained in the quantification experiments. The results indicate that the IPC C_T values for the samples tested did not increase significantly compared to that for the NTC. Thus, the PCR inhibitors present in blood, blood spiked with inhibitor mix, dyes from denim, and those arising from the exposure to the environment have been efficiently removed during the extraction of DNA using the PrepFiler™ Kit. Similar results were obtained for

TABLE 2—*Stability studies: average concentration of DNA in the extracts.*

Sample*	Donor 1		Donor 2	
	Concentration (ng/µL)	SD	Concentration (ng/µL)	SD
BSCI	5.00	0.37	4.85	0.28
BSD	3.86	0.33	3.30	0.30
BSC7	8.68	0.87	7.33	0.45

*BSCI, blood (5 µL spiked with inhibitor mix) stain on noncolored cotton cloth; BSD, blood (5 µL) stain on denim; BSC7, blood (10 µL) stain on noncolored cotton cloth exposed to outdoor environment for 7 days. The samples were extracted in triplicate and each extract was quantified in duplicate. A 5-mm punch size was used in all cases.

SD, standard deviation.

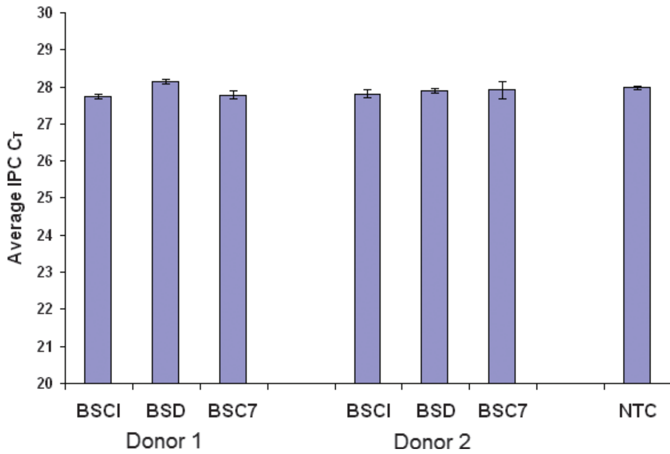

FIG. 3—*Stability studies: average IPC C_T values for three different sample types with chemical or environmental insults. BSCI, blood (5 μL spiked with inhibitor mix) stain on noncolored cotton cloth; BSD, blood (5 μL) stain on denim; BSC7, blood (10 μL) stain on noncolored cotton cloth exposed to outdoor environment for 7 days. The samples were extracted in triplicate and each extract was quantified in duplicate.*

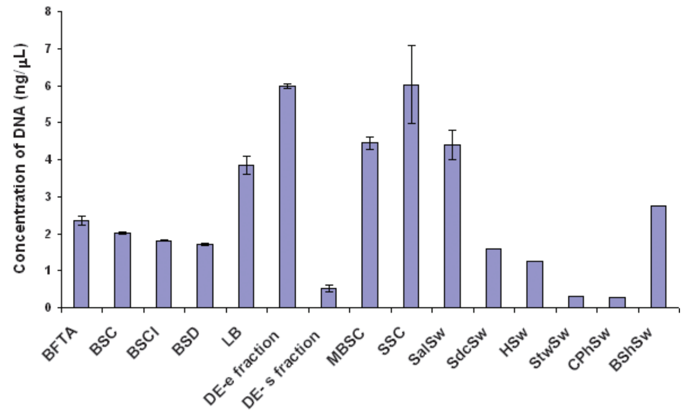

FIG. 4—*Case-type samples: average concentration of DNA in the extract obtained from various samples. BFTA, blood (2 μL) stain on FTA® paper; BSC, blood (2 μL) stain on noncolored cotton cloth; BSCI, blood (2 μL spiked with inhibitor mix) stain on noncolored cotton cloth; BSD, blood (2 μL) stain on denim; LB, liquid blood (2 μL); DE-e fraction, epithelial cell fraction; DE-s fraction, sperm fraction; MBSC, mixed blood (1 and 4 μL blood from male and female donors, respectively) stain on noncolored cotton cloth; SSC, semen (1 μL) stain on noncolored cotton cloth; SalSw, saliva (50 μL) on swab; SdcSw, swab of a soda can; HSw, swab of a hat; StwSw, swab of a steering wheel; CPhSw, swab of a cell phone; BShSw, swab of blood stain on shoe. Samples BFTA, BSC, BSCI, BSD, DE-e fraction, DE-s fraction, MBSC, SSC, and SalSw were extracted in duplicate and each extract was quantified in duplicate. Standard deviations of four quantification values are represented by the error bars for these samples. Samples SdcSw, HSw, StwSw, CPhSw, and BShSw were extracted in single replicate and each extract was quantified in duplicate. Quantification values are the averages of the two replicates.*

extraction blanks (data not shown) confirming that the PrepFiler™ Kit reagents themselves did not introduce PCR inhibitors during the extraction procedure.

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™ Kit. Forensic-type samples were prepared using different substrates and saliva, blood, and semen obtained from human donors as described in Materials and Methods. Figure 4 summarizes the results for the different sample types investigated. The average concentration of DNA in the extract obtained from 2 μL of LB was as high as 3.85 ng/μL. Similarly, the concentration of DNA in the extract obtained from different blood stain samples prepared using 2 μL of blood ranged between 1.72 and 2.35 ng/μL. This varied result was expected because not all cells in a stained sample are accessible to the lysis buffer, and cells may be entrapped within the substrate and/or bound to the matrix. Despite the low contents of biological material, sufficient quantity of DNA was obtained from all samples investigated including touch evidence. The IPC C_T values for all case-type samples studied were similar to that obtained for NTC indicating effective removal of PCR inhibitors from the biological samples and substrates (data not shown). The results demonstrate that the PrepFiler™ Kit is efficient and useful for genomic DNA extractions from forensic case-type samples.

TABLE 3—*Correlation study: total DNA yield (ng) for commonly used DNA extraction methods.*

	PrepFiler™		DNA IQ™		Ph-Ch[†]		EZ1[‡]		Micro[§]	
Sample*	R1[¶]	R2**	R1	R2	R1	R2	R1	R2	R1	R2
BFTA	76.5	78.0	21.0	21.5	19.5	36.0	20.0	22.5	10.0	19.0
BSC	60.0	107.0	46.5	38.0	67.5	38.5	29.5	34.5	17.5	23.5
BSCI	74.0	82.5	29.0	33.0	37.5	33.5	33.0	22.0	19.5	18.0
BSD	66.0	57.0	26.5	25.0	57.5	69.5	20.5	21.5	19.0	25.0
SSC	161.0	196.5	70.5	66.5	84.0	74.0	87.0	89.0	49.5	55.0
SalSw	149.5	160.0	11.0	19.0	53.5	72.0	36.0	38.5	15.5	14.5
LB	94.5	89.5	29.5	38.5	30.0	30.0	51.5	42.0	26.5	30.5

*BFTA, blood (2 μL) stain on FTA® paper; BSC, blood (2 μL) stain on noncolored cotton cloth; BSCI, blood (2 μL spiked with inhibitor mix) stain on noncolored cotton cloth; BSD, blood (2 μL) stain on denim; SSC, semen (1 μL) stain on noncolored cotton cloth; SalSw, saliva (50 μL) on swab; LB, liquid blood (2 μL). A 5-mm punch size was used for stains and whole swabs were used for swab samples. Each sample was extracted in two replicates as indicated. All samples were quantified in single replicates on one plate in order to avoid experiment to experiment error.

[†]Phenol–chloroform.

[‡]EZ1 DNA Investigator Kit.

[§]QIAamp® DNA Micro Kit.

[¶]Replicate 1.

**Replicate 2.

TABLE 4—*Correlation studies: average IPC C_T values.*

Sample*	PrepFiler™	DNA IQ™	Ph-Ch†	EZ1‡	Micro§
BFTA	27.72	27.73	27.91	28.06	28.02
BSC	27.58	27.53	27.73	27.95	27.88
BSCI	27.60	27.48	27.83	28.00	27.84
BSD	27.85	27.65	27.82	28.06	27.99
SSC	27.63	27.72	27.92	27.98	27.87
SalSw	27.71	27.63	27.85	27.90	27.76
LB	27.84	27.70	27.89	27.96	27.79
XB	27.77	27.79	27.97	28.08	27.90
NTC	28.01	–	–	–	–

*BFTA, blood (2 μL) stain on FTA® paper; BSC, blood (2 μL) stain on noncolored cotton cloth; BSCI, blood (2 μL spiked with inhibitor mix) stain on noncolored cotton cloth; BSD, blood (2 μL) stain on denim; SSC, semen (1 μL) stain on noncolored cotton cloth; SalSw, saliva (50 μL) on swab; LB, liquid blood (2 μL); XB, extraction blank; NTC, nontemplate control. Each sample was extracted in duplicate. All samples were quantified in single replicate on one plate in order to avoid experiment to experiment error. The C_T values provided are an average of two values for each sample type.

†Phenol–chloroform.

‡EZ1 DNA Investigator Kit.

§QIAamp® DNA Micro Kit.

Contamination Studies

To monitor contamination, each study included extraction blanks. A total of 20 extraction blanks were processed throughout the validation studies over a 3-month period. None of the extraction blanks exhibited detectable quantities of genomic DNA and none produced STR profiles when amplified. Thus, the reagents and operations of the PrepFiler™ Kit did not introduce any detectable contamination of human DNA.

Correlation Studies

The goals for the efficient extraction of DNA are to obtain the DNA in concentrated form, in maximum quantity, and with high purity. The quantity and extent of purity of isolated DNA may vary based on the extraction method used. The performance of the PrepFiler™ Kit was compared with the traditional phenol–chloroform method and commonly used commercial methods, by processing the forensic-type samples described in the Materials and Methods. Efforts were made to have uniform sample inputs. Parameters such as yield of DNA, concentration of DNA, and the presence of PCR inhibitors were assessed. Total yield of DNA in the extract is summarized in Table 3. The yield of DNA in the extracts obtained using the PrepFiler™ Kit was higher for all sample types investigated compared to the other extraction methods evaluated; the DNA yields for the BSD samples obtained with the PrepFiler™ Kit and phenol–chloroform were similar. The concentration of DNA in the extract obtained with the PrepFiler™ Kit was also higher for all samples tested, except for the BSD samples (data not shown) compared with other methods examined. The elution volume for all sample types and DNA extraction methods investigated remained constant at 50 μL. Extracts containing higher concentrations of DNA require input of a lower extract volume in the amplification reaction. A lower concentration of DNA requires a higher volume of extract to be used for amplification. In such a case, a larger amount of inhibitors and salts are also transferred into the STR amplification reaction which may result in higher peak imbalances for heterozygous loci, lower amplitude for loci with longer amplicons, or poor interlocus balance. Thus, obtaining DNA extract containing higher concentration of DNA is desired.

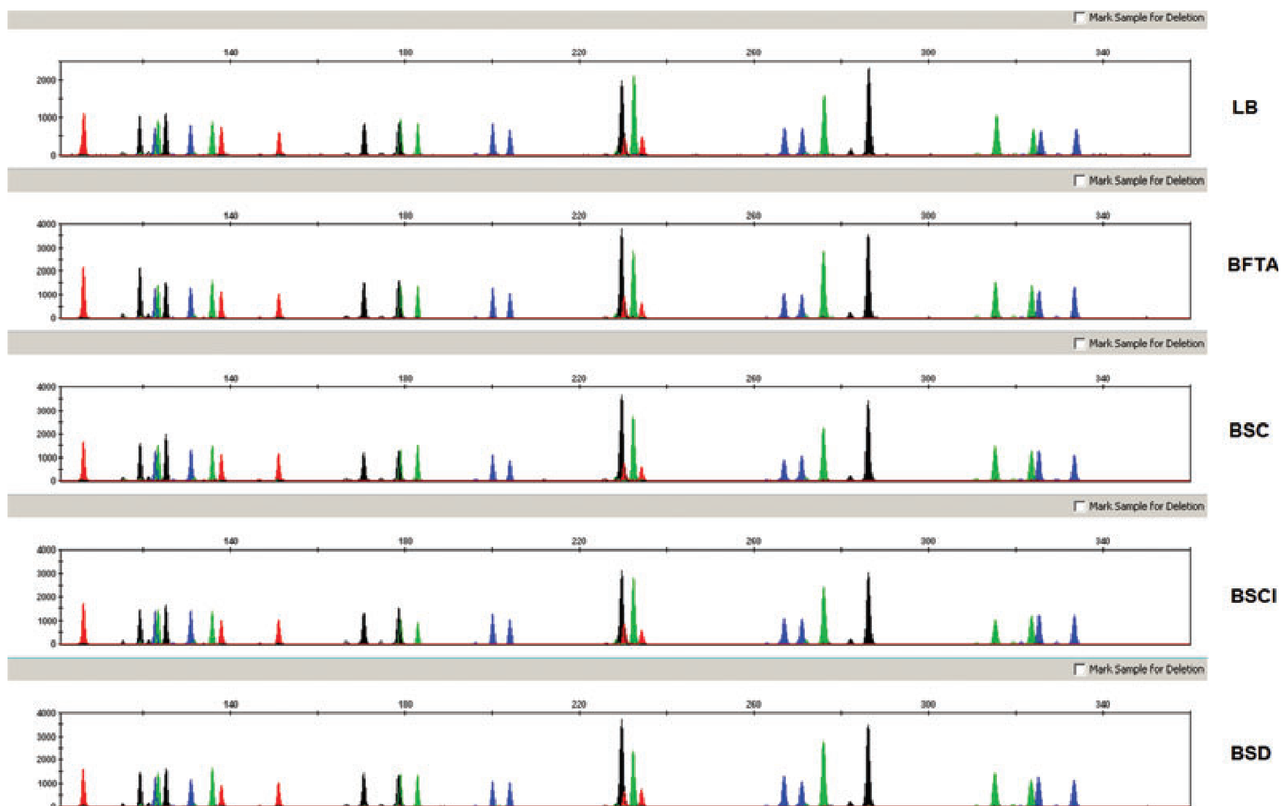

FIG. 5—*Identifiler® profiles for different sample types. LB, liquid blood (2 μL); BFTA, blood (2 μL) stain on FTA® paper; BSC, blood (2 μL) stain on noncolored cotton cloth; BSCI, blood (2 μL spiked with inhibitor mix) stain on noncolored cotton cloth; BSD, blood (2 μL) stain on denim. Y-axis represents rfu (relative fluorescence units) values and X-axis represents size estimates of the fragments in bases.*

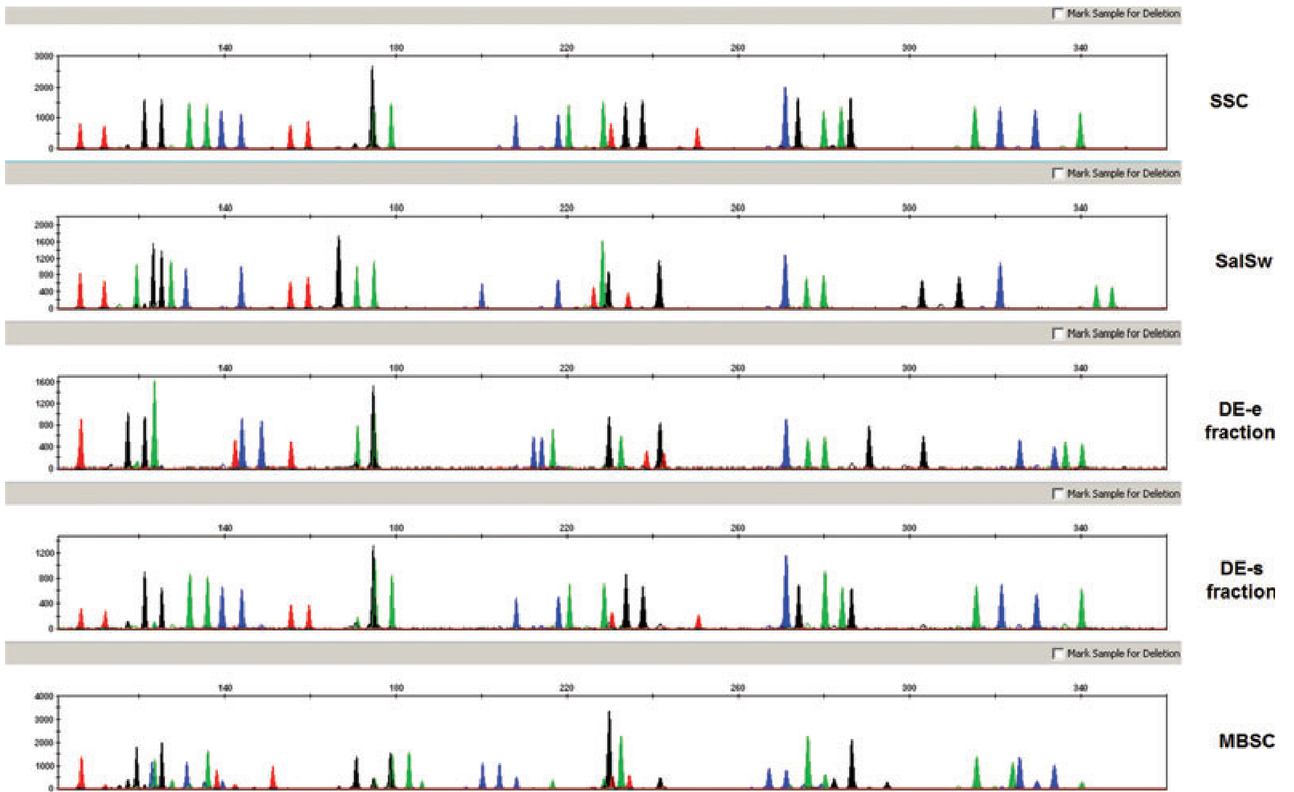

FIG. 6—*Identifiler® profiles for different sample types. SSC, semen (1 μL) stain on noncolored cotton cloth; SalSw, saliva (50 μL) on swab; DE-e fraction, epithelial cell fraction; DE-s fraction, sperm fraction; MBSC, mixed blood (1 and 4 μL blood from male and female donors, respectively) stain on noncolored cotton cloth. Y-axis represents rfu (relative fluorescence units) values and X-axis represents size estimates of the fragments in bases.*

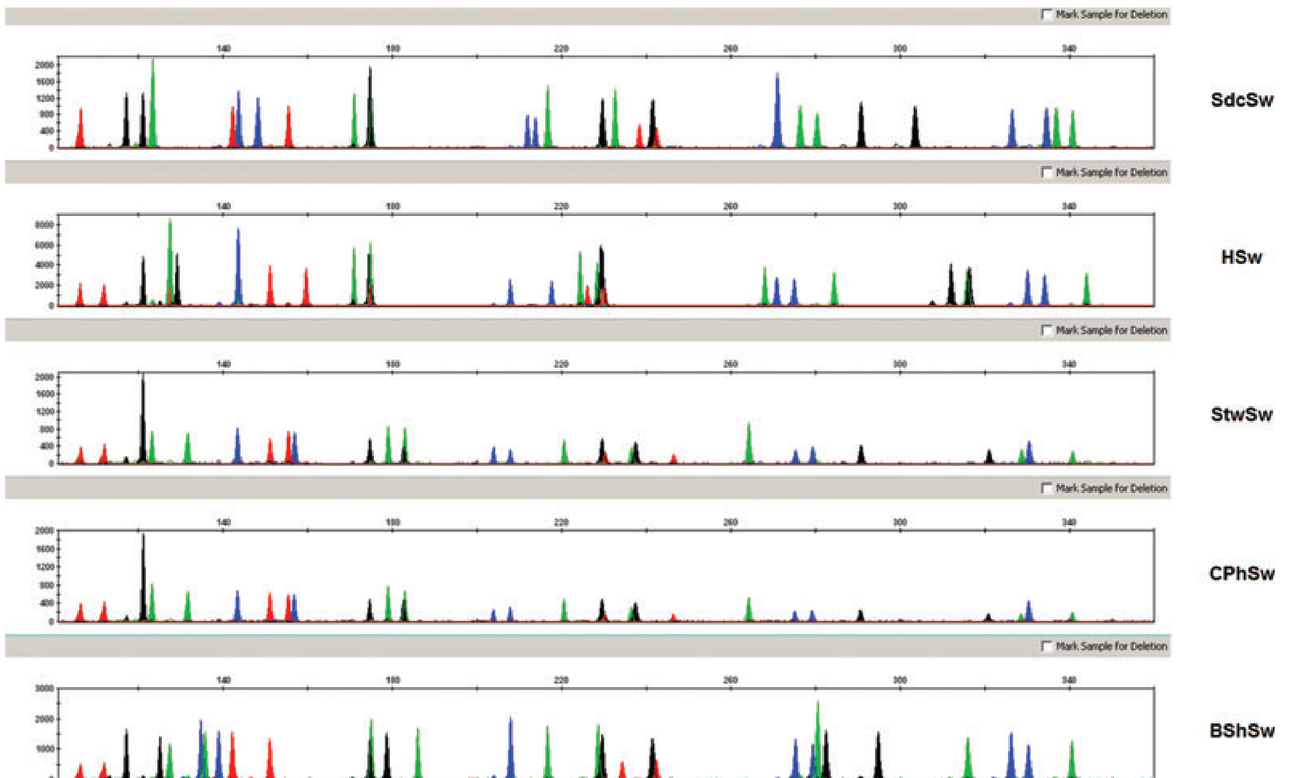

FIG. 7—*Identifiler® profiles for different sample types. SdcSw, swab of a soda can; HSw, swab of a hat; StwSw, swab of a steering wheel; CPhSw, swab of a cell phone; BShSw, swab of blood stain on shoe. Y-axis represents rfu (relative fluorescence units) values and X-axis represents size estimates of the fragments in bases.*

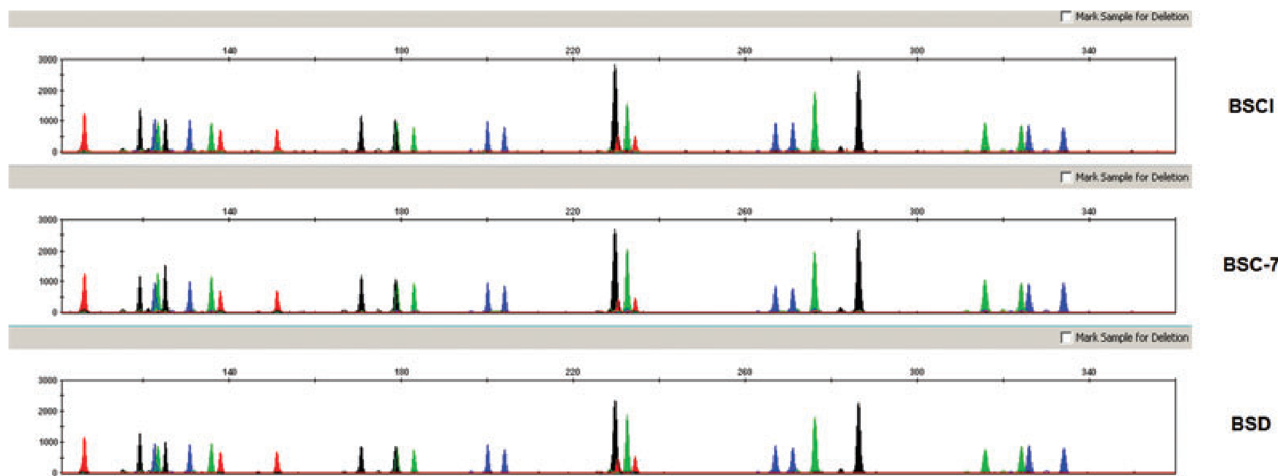

FIG. 8—*Identifiler® profiles for the compromised samples included in the stability studies. BSCI, blood (5 μL spiked with inhibitor mix) stain on noncolored cotton cloth; BSC-7, blood (10 μL) stain on noncolored cotton cloth exposed to outdoor environment for 7 days; BSD, blood (5 μL) stain on denim. Y-axis represents rfu (relative fluorescence units) values and X-axis represents size estimates of the fragments in bases.*

The IPC C_T values for all sample types extracted using different extraction chemistries were similar to those obtained for the NTC indicating that the PCR inhibitors were efficiently removed (Table 4).

STR Analysis

Although the extraction of DNA is the first step in the forensic DNA analysis, the ultimate goal is to produce a high-quality STR profile. The quality of isolated DNA using PrepFiler™ Forensic DNA Kit was further judged by the nature of STR profiles generated using the Identifiler® Kit. The Identifiler® profiles for different forensic case-type samples are presented in Figs. 5–7. Similarly, Identifiler® profiles for the compromised samples used in the stability studies are presented in Fig. 8. The STR profiles from the case-type and compromised samples were complete, conclusive, and devoid of PCR artifacts. The data demonstrates that the PrepFiler™ Kit is effective in isolating high-quality DNA from biological samples that are commonly observed in forensic casework.

Conclusions

The PrepFiler™ Forensic DNA Extraction Kit is developed for the extraction of genomic DNA from biological samples that are commonly observed in the forensic DNA laboratory. DNA from the cell lysate binds reversibly with the magnetic particles and remains bound under the wash conditions that eliminate the inhibitors of PCR and other such substances. The DNA from the magnetic particles is then released in the elution buffer. The extracted DNA thus obtained is free of detectable PCR inhibitors. Utility of the PrepFiler™ Kit is demonstrated using forensic-type samples including inhibited samples and samples that faced chemical and environmental insults. The yields of DNA for the different forensic-type samples tested were greater than those obtained by the widely used phenol–chloroform method. In conclusion, we have developed a reliable method for obtaining high-quality genomic DNA from biological samples that is suitable for subsequent real-time PCR and STR profiling applications. The PrepFiler™ Kit is amenable to automation. The studies on optimization of the automation scripts using Tecan EVO® 150 (Tecan Schweiz AG, Mannedorf, Switzerland) platform are in progress.

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