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TECHNICAL NOTE

CRIMINALISTICS

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New Optimized DNA Extraction Protocol for Fingerprints Deposited on a Special Self-Adhesive Security Seal and Other Latent Samples Used for Human Identification*

ABSTRACT: Obtaining complete short tandem repeat (STR) profiles from fingerprints containing minimal amounts of DNA, using standard extraction techniques, can be difficult. The aim of this study was to evaluate a new kit, Fingerprint DNA Finder (FDF Kit), recently launched for the extraction of DNA and STR profiling from fingerprints placed on a special device known as Self-Adhesive Security Seal Sticker® and other latent fingerprints on forensic evidentiary material like metallic guns. The DNA extraction system is based on a reversal of the silica principle, and all the potential inhibiting substances are retained on the surface of a special adsorbent, while nucleic acids are not bound and remain in solution dramatically improving DNA recovery. DNA yield was quite variable among the samples tested, rendering in most of the cases (>90%) complete STR profiles, free of PCR inhibitors, and devoid of artifacts. Even samples with DNA amount below 100 pg could be successfully analyzed.

KEYWORDS: forensic science, DNA typing, fingerprint processing, epithelial cells, short tandem repeat, DNA extraction

Using modern short tandem repeat (STR) genotyping technologies, it is possible to obtain DNA profiles from very small amounts of nucleic acids (1,2). A single skin contact can transfer enough DNA for successful STR typing (3–5). Kinga Balogh et al. (6) were even able to get complete STR profiles and mtDNA sequences from latent fingerprints on paper.

Thus, fingerprints are a possible DNA source and become more and more important in forensic DNA investigations, like casework and databasing, but it is still difficult to get complete and reliable STR profiles.

Numerous modifications have been applied to improve standard STR profiling of forensic DNA samples. For example, Roeder et al. (7) demonstrated that the use of higher number of cycles and increased injection time during capillary electrophoresis can maximize the profiling success of samples with suboptimal DNA quantities. Smith and Ballantyne (8) also demonstrated that post-polymerase chain reaction (PCR) purification of the PCR product can increase the sensitivity of capillary electrophoresis to such an extent that DNA profiles can be obtained from <100 pg of DNA using 28-cycle amplification.

Nevertheless, particular attention should be given to the sample collection and the DNA extraction, as these are the first critical steps of successful DNA profiling (9). Many commercial DNA extraction kits are based on the binding of DNA and removal of inhibitors by several washing steps. The multiple steps cause the loss of some DNA, and thus, DNA extracted from many forensic samples falls below the kit manufacturer's specified concentrations either because there is not enough total DNA in the extract or it is so dilute that not enough volume of the extract can be added to the PCR (8). Schiffner et al. (10) developed a one-step sample digestion and purification protocol and found out that the extraction efficiency for low-level samples can be increased by using protocols with fewer steps.

The aim of our study was to evaluate the recently launched Fingerprint DNA Finder (FDF Kit) for the extraction of DNA and STR profiling from (i) fingerprints placed on a special device known as Self-Adhesive Security Seal Sticker® (11,12) and (ii) latent fingerprints on forensic evidentiary material.

The FDF Kit is particularly suited for the extraction of DNA from samples with limited quantities of nucleated cells as it is based on a simple one-step protocol. The Fingerprint Sticker® can retain epidermal cells when the skin is imprinted on the acrylic layer and thus can be used as a simple sample collection tool for the setup of DNA profile databases for human identification purposes.

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Materials and Methods

Sample Preparation

Fingerprints were applied on the following seal and surfaces:

- Self-Adhesive Security Seal Sticker® (Fig. 1): A clean fingerprint was obtained by pressing a thumb or an index finger on the intermediate polypropylene film layer, treated with an adhesive acrylate polymer, and covered immediately with the upper cover until DNA extraction. No previous hand cleaning treatment was applied. Single fingerprints were obtained from 146 different donors.
- Latent fingerprints: Samples were collected from four different Bersa™ 9 mm pistols (Bersa S.A., Buenos Aires, Argentina) and one Smith & Wesson™ 357 magnum revolver (Springfield, MA).

After cleaning the weapons of existing fingerprints (bleach and ethanol treatment), the firearms were manipulated (loaded) by the proband and fired into a ballistic tube device. After shooting, samples were collected from the fired cartridge case, trigger, magazine, and slide barrel (pistols) or fired bullet case, trigger, and hammer (revolver) by using cotton swabs. The whole process was repeated three times over each gun.

DNA Isolation

DNA isolation was carried out using the FDF Kit (patent in progress) recently developed by BioSystems S.A. (Buenos Aires, Argentina) and Nexttec GmbH (Leverkusen, Germany).

Lysis buffer was freshly prepared by mixing 71.4 μL of buffer FP 1, 2 μL of buffer FP 2, and 6.8 μL of buffer FP 3, included in the FDF Kit.

A total volume of 30 μL of lysis buffer was pipetted either directly on the adhesive layer of the seal with the fingerprint or on the head of a DNA-free standard cotton swab with wooden shaft to collect the latent fingerprint on the guns (sample collection from the adhesive layer of the seal with isopropanol containing foam swabs was also tested for STR profiling, but with no reproducible results; not shown).

The fingerprint on the seal or firearm was swabbed gently, with circular movements to cover the entire surface and while rotating the swab head. Each component of the gun was swabbed using different swabs.

The cotton head of the swab (including a small piece of the wooden shaft) was immediately placed into a 1.5-mL plastic tube, and an additional volume of 50 μL of the lysis buffer was added.

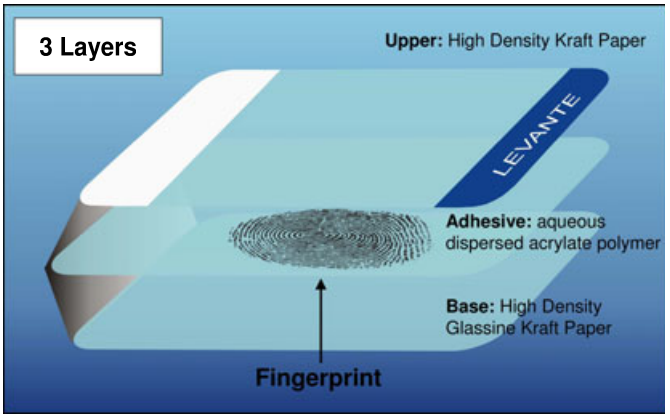

FIG. 1—*A schematic representation of the Self-Adhesive Security Seal Sticker®, composed of several layers, including polypropylene film and dispersed acrylate adhesive. The lower side of the upper layer contains a paraffin film that prevents adhesion to the acrylate.*

Lysis was performed by incubation at 60°C in a thermomixer with shaking at 600 rpm for 3 h.

After incubation, the swab head was transferred to a plastic "spin basket" with a collection tube and centrifuged at maximum speed (15,800 × *g*) for 1 min.

The resulting centrifuged liquid extracted from the swab was mixed with the remaining liquid from the incubation tube. The final volume was always about 55–60 μL.

The mixture was transferred to a previously equilibrated Nexttec clean column (according to the FDF Kit protocol) and centrifuged for 1 min at 750×*g* after incubation for 3 min at room temperature.

The eluate contained the purified DNA, while other molecules, such as potential PCR inhibitors, were retained by the chemically modified silica within the column.

DNA Quantification

DNA concentrations were determined using the Applied Biosystems Quantifiler™ Human DNA Quantification kit (Life Technologies, Inc., Carlsbad, CA), which can also reveal the presence of PCR inhibitors as the kit includes an internal positive control.

DNA Amplification and Profile Analysis

STR amplification was performed using the AmpFlSTR® Identifiler® PCR Amplification Kit, on a GeneAmp® 9700 thermal cycler (Life Technologies, Inc.), according to manufacturer's instructions. Electrophoresis was performed on an Applied Biosystems 3130 Genetic Analyzer and analyzed using GeneMapper® *ID* v 3.2.1 software. The threshold for calling peaks in the analysis method was set to 50 relative fluorescent units (rfu).

Post-PCR Purification

The initial volume containing the PCR products (25 μL) was purified and concentrated using the Qiagen MinElute PCR Purification kit (Qiagen GmbH, Hiden, Germany). following manufacturer's instructions. Cleaned PCR product was eluted in 10 μL of elution buffer resulting in *c.* 2.5× concentration of the PCR product.

Results

Results Using the Self-Adhesive Security Seal Sticker®

To evaluate the shedder index defined by Murray et al. (13) as the tendency of an individual to leave his/her DNA on a touched surface, a total of 146 DNA samples from fingerprints were quantified and also subjected to STR analysis. Figure 2 shows the results of the different ranges of DNA mass obtained in the total sample population.

From the STR analysis of 146 fingerprint samples, 120 samples (82.2%) could be amplified and genotyped successfully, 16 samples (11%) showed partial profiles (<16 STRs, but always more than 10 markers), and only 10 samples (6.8%) showed either operator-induced contaminations or did not contain DNA. Contributors of the samples were male and female (almost equally distributed), and there were no significant gender-related differences (data not shown). A typical STR profile is shown in Fig. 3.

To improve the results for samples with incomplete or low profile (low rfu), and thus without automatic allele calls, the PCR products were additionally purified with the Qiagen MinElute PCR Purification kit. Results are shown in Table 1. From 16 samples, the STR profile could be completed in 12 (75%). Figure 4 shows

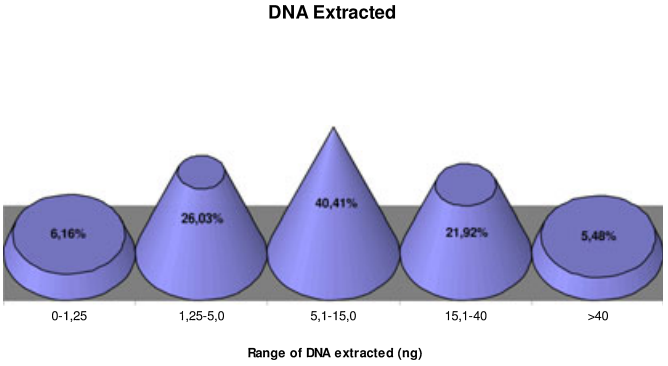

FIG. 2—*Estimation of DNA mass obtained from the fingerprints deposited over the Self-Adhesive Security Seal Sticker®. The X-axis shows arbitrary ranges of DNA amount and the percentage represents the number of samples that are within each range.*

partial electropherograms of a sample with and without post-PCR purification (only four STRs are shown).

Results from Latent Fingerprints from Guns

Fingerprint samples were recovered from several different gun components, such as magazine, trigger, and slide barrel (pistols), trigger and hammer (revolver), as well as from fired bullet case.

Figure 5*A* shows simultaneously the profiles from a trigger, magazine, and slide barrel of a representative gun (only four STRs are shown). All three electropherograms showed the same profile, which also corresponded to the proband (not shown). However, as was already described (14), the profile was altered in the fired cartridge case (not shown). Similar results were obtained with all guns tested and with all replicate samples from the same gun.

TABLE 1—*Results of samples with incomplete profile analyzed after a post-PCR purification step.*

	Results After Post-PCR Purification Step	
Incomplete or Low Profile	Total Recovery	Partial Recovery
16 (100%)	12 (75%)	4 (25%)

Qiagen MinElute PCR Purification kit was used for post-PCR purification step.

DNA profiles with peaks below the amplitude threshold of 50 rfu were purified and concentrated with a post-PCR purification method (Qiagen MinElute PCR Purification kit). Figure 5*B* shows the electropherograms of the purified PCR reactions (only four STRs are shown). Peaks are four times higher compared to the unpurified PCR reaction and no drop-in or drop-out effects were detected.

No PCR inhibitors were found either in the samples taken from Fingerprint Stickers® or from the metallic guns (data not shown).

Discussion

We describe herein the use of an innovative new DNA extraction kit, the FDF Kit, for a one-step purification of genomic DNA

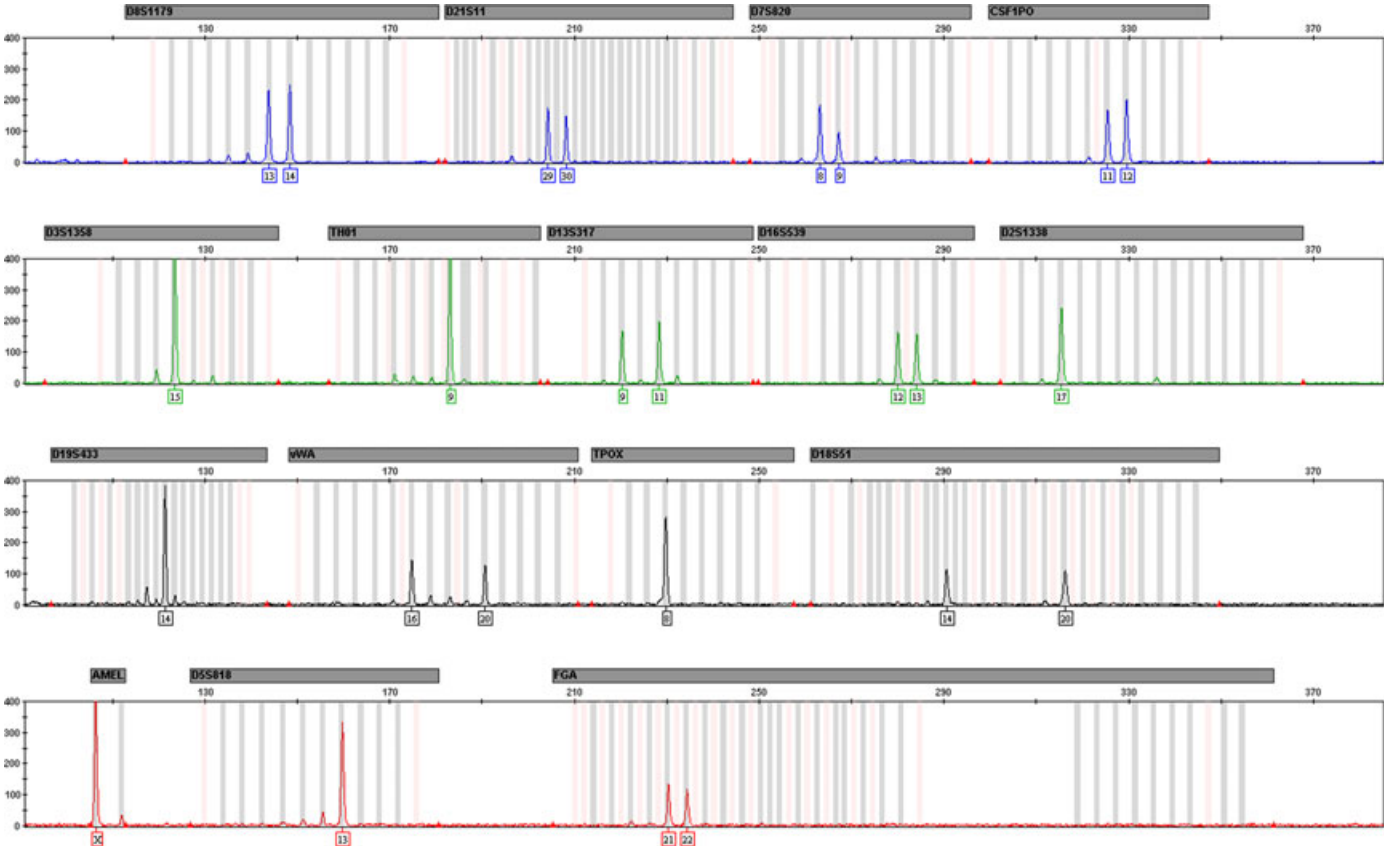

FIG. 3—*Typical short tandem repeat result using a fingerprint with low DNA amount (final DNA concentration of the sample = 24 pg/μL). The entire 16 markers profile is shown.*

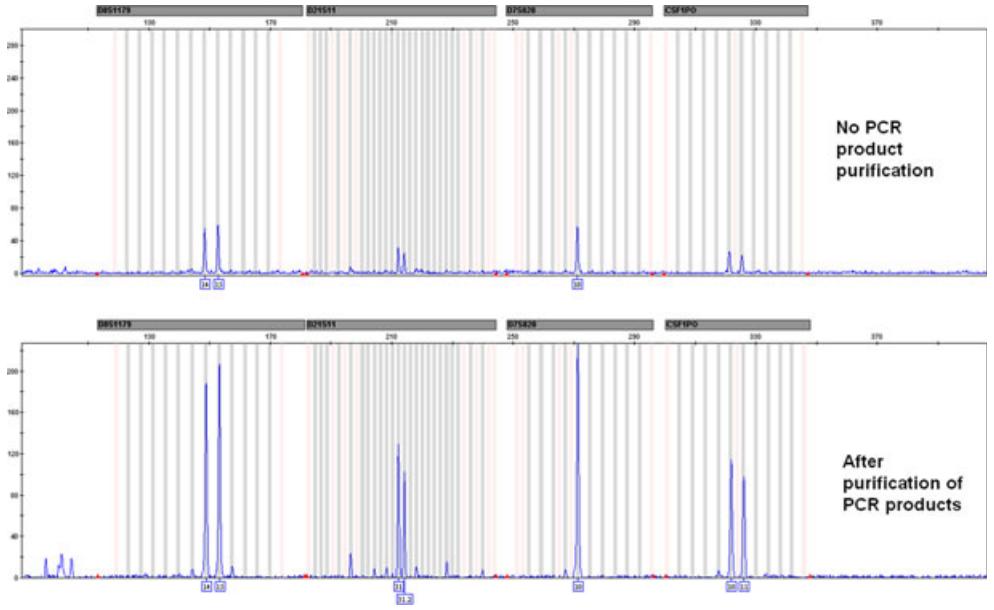

FIG. 4—*Improvement of the short tandem repeat profile using a post-PCR purification protocol (Qiagen MinElute PCR Purification kit). Only D8S1179, D21S11, D7S820, and CSF1PO are shown.*

for STR analysis, either from biological samples left on a special seal device used for fingerprint purposes (the Self-Adhesive Security Seal Sticker®) or from latent fingerprints left on metallic guns.

Nexttec's DNA extraction system is based on a reversal of the well-known silica principle. After a simple and effective lysis procedure, the inhibiting substances like proteins and low molecular weight components are retained on the surface of a special adsorbent, while nucleic acids are not bound (15). The purified DNA is free of PCR inhibitors and ready for downstream applications such as real-time PCR and STR analysis. As has been reported by Lauk and Schaaf (16), the Nexttec technology is well suited for forensic DNA extraction from stamps (saliva) followed by STR profiling.

By improving the buffers and the protocol of the Nexttec extraction system, we were able to obtain DNA from fingerprints (epidermal cells) with a very simple extraction protocol and without using more PCR cycles, which usually induce increased imbalance of heterozygotes and is associated with an increase in the size (peak area) of stutters (17).

Our previous experiments using the same samples and standard organic solvent procedures or resins (Chelex) showed inconsistent results (incomplete profiles, PCR inhibitors). Since using the new protocol, all this issues were successfully solved.

Furthermore, in our experiments, the obtained STR profiles could be analyzed automatically with the GeneMapper® *ID* v 3.2.1 software. In most of the cases, there was no spurious marker identification. Comparison of STR profiles from the fingerprints with profiles from buccal swab samples from the same persons showed 100% concordance.

Thus, our results show that it is possible to obtain and type DNA from single fingerprints. Also samples with a DNA mass lower than 100 pg rendered clear profiles, some of them with low peak height, but with a clean baseline, that allows the software to perform automatic identification of the STR profile. Very low signals can be improved by purification of the PCR products using commercial silica-based PCR purification kits.

We have avoided the use of the term "low copy number" (LCN) samples, as we assumed that LCN is any sample that contains less than 100 pg of template DNA, or more precisely, LCN typing is better defined as the analysis of any results below the stochastic threshold for normal interpretation (9). As a result of large variation in the amount of DNA in our samples, some fall into that category, but most of them should not be considered LCN.

Regarding the use of the DNA profiles obtained from the fingerprints deposited on the Fingerprint Sticker®, the results demonstrate the feasibility of using this kind of samples as a DNA source to construct databases.

The method permits:

- The use of noninvasive sampling technique (different from blood or buccal swabs).
- The handling of samples without biological risk.
- The storage of samples without bacterial contaminations as the DNA is conserved in a dry condition, protected from the environment (further studies must be performed to evaluate long storage conditions without DNA alteration).
- The transport of large numbers of samples in small containers, avoiding the necessity for special transport conditions.
- The association of the DNA source with the personal data of the proband (i.e., fingerprint image stored in an automated fingerprint identification system), under proven security conditions similar to a barcode on the same sheet of paper.

In previous experiments (not shown), we found no DNA contamination on fingerprints deposited on stickers from donors that previously "shake hands" with other people, but more work should be carried out to extensively confirm this previous finding.

Further studies should be conducted to compare DNA extraction using the FDF Kit with other procedures involving several adsorption/elution steps. Even assuming similar results, the simplified one-step protocol used by the FDF Kit avoids time-consuming and labor-intensive experiments, allowing easy laboratory automation (plate format) and low operation costs.

Considering fingerprints left on guns, it is well known that the best place to locate latent samples is on the smooth surface of the barrel, the magazine, or cartridge case, making our technique ideal for this application.

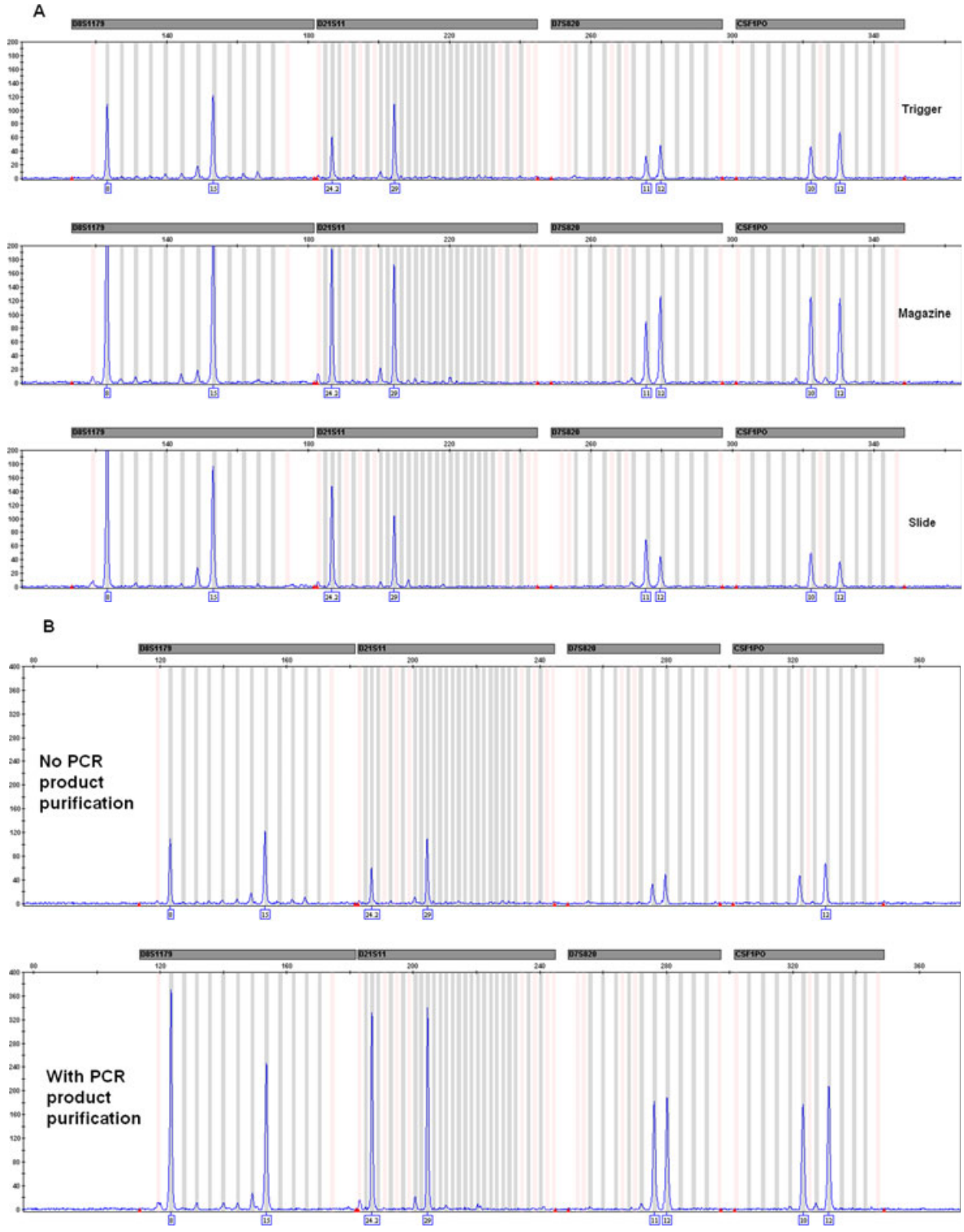

FIG. 5—*(A) Typical electropherogram of samples taken from a Bersa™ 9 mm pistol; trigger with 79 pg DNA/μL, magazine with 148 pg DNA/μL, and slide with 252 pg DNA/μL. (B) Electropherogram of the trigger of the gun analyzed in (A) after purification of the PCR products, obtaining an improvement of four times in the peak heights. Only D8S1179, D21S11, D7S820, and CSF1PO are shown.*

Conversely, DNA-containing material is often found on rough surfaces where cells are rubbed off or collected in low spots; therefore, we chose the analysis of the trigger, the rough part of the slide or the magazine (pistols), or the hammer (revolver), where the profiles were always coincident with those obtained from the other parts.

In our hands, the profiles obtained from the fired cartridge case rendered altered profiles compared with those obtained from the gun. However, these results are not reliable. The allele peaks are near or below the amplitude threshold of 50 rfu and should therefore be interpreted very carefully. As previously described by Horsman-Halla et al. (14), these effect can be explained, among other

reasons, by the high temperature reached during the firing process (data not shown). Further detailed studies of STR profiling from fired cartridge cases are necessary.

It is also well known that when a gun is recovered from the crime scene and booked in for evidence to match with a fired gun or eyewitness identification, it is usually handled by the grip or nonsmooth surfaces, preserving the smooth ones for latent fingerprint detection. Our results, however, demonstrated that the rough parts of the gun are ideal for DNA analysis, and therefore, those areas must also not be handled to preserve genetic evidence.

Thus, the advantage of this technique is also that the same gun can be used both for fingerprint and for DNA analysis, allowing both types of evidence to be obtained from the same gun, but this requires careful handling at the crime scene and afterward.

We conclude that the FDF Kit is suitable for multiple types of forensic samples. The simple and fast one-step protocol of the FDF Kit combines the effective removal of PCR inhibitors with appropriate yield of DNA. Thus, valid and highly informative DNA profiles can be obtained from DNA sources like fingerprints on acrylic adhesive surfaces or traces with limited quantities of DNA for database construction and forensic casework, respectively.

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