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A customized protocol to generate STR profiles from latent fingerprints

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ABSTRACT

For decades, dactyloscopical and DNA analyses have both played a key role in forensic investigations involving friction skin patterns and/or human biological material. In many occasions, friction ridge impressions may hold little discriminatory power due to low quality of ridge patterns and/or insufficient area extension of such patterns. In these cases, an appropriate human DNA collection and a high-quality DNA extraction become crucial steps to yield a genetic identity from an unsuitable latent fingerprint pattern. Indeed, over the past few years, it has been proven that complete Short Tandem Repeat (STR) profiles can be obtained from a touch DNA sample. In this study, a protocol has been customized to maximize the performance of genetic profiling from latent fingerprints. Six participants provided two sets of finger impressions on pre-cleaned glass surfaces. These impressions were generated by the participant's dominant (DH) and non-dominant hand (NDH). Genetic material from fingerprints was pooled using a cotton swab for each donor and hand, combining 1–5 depositions consecutively. This was followed by DNA extraction, Real-Time PCR for DNA quantification, capillary electrophoresis (CE) for sequencing, and genotyping software for STR profiling. DNA yield was measured by ng/cm^2 (DNA/fingerprint area). Statistical tests detected DNA yield differences by donor's sex, age distribution, handedness and fingerprint pooling. Results revealed that DNA quantities from DH was dependent on the number of pooled fingerprints. However, NDH yielded similar DNA quantities across all fingerprint pooled combinations. With the aid of a customized protocol, DNA titers was improved and meaningful STR profiles were produced for donors' DH and NDH.

1. Introduction

Friction skin ridge patterns are considered one of the most relevant physical evidence, with donor identification potential, that can be recovered from crime scenes. Indeed, for a century, fingerprints have been examined to exclude or individualize a suspect in crime-related investigations. Friction skin may not only provide a unique ridge pattern through the location, orientation, and type of minutiae but also contain biological material; both items useful for identification purposes. Often, crime investigators must choose between enhancing a latent fingerprint or extracting its DNA [1]. In many occasions, fingerprint patterns may not be valuable as evidence because of insufficient quality of ridge patterns. These situations make DNA analysis the only alternative for donor identification.

Generally, surfaces where fingerprints are collected are classified into two main classes: porous and non-porous. Those deposited onto porous surfaces are absorbed into the substrate and tend to be more durable and resilient to external insults [2]. Non-porous surfaces are non-absorbent, making fingerprints more susceptible to damage as the residue existing on the outermost surface is more exposed to environmental and mechanical influences [3]. The main objective was to develop a protocol for human DNA collection and a high-quality extraction to produce a meaningful genetic identity from an unsuitable fingerprint pattern considering biological sex, age of the donor, handedness, and fingerprint pooling.

2. Materials and methods

Six volunteers (3 males and 3 females) of two age groups (20–40 and 50–70 years old) agreed to participate in the research by donating their fingermarks and their DNA. After signing and agreeing with the consent form, volunteers gently washed their hands for one minute with water

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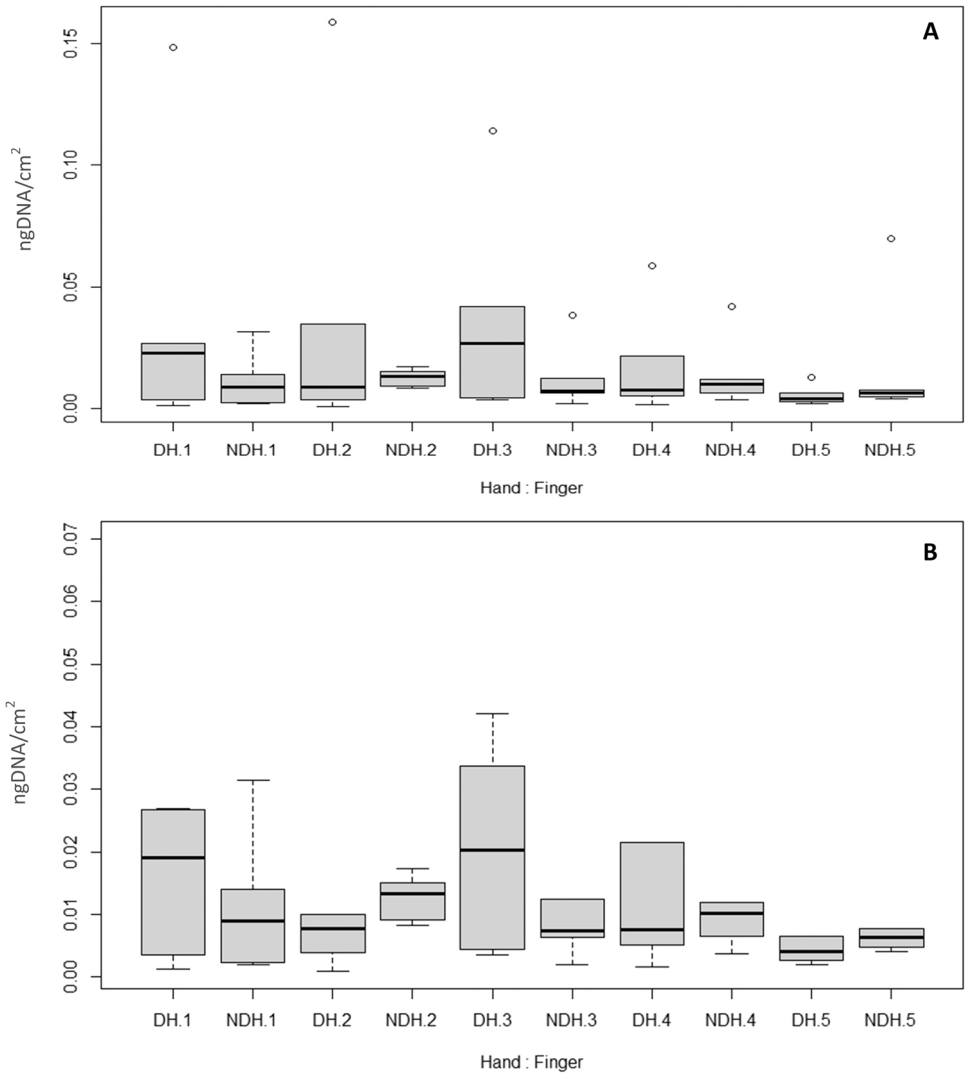

Fig. 1. Human DNA obtained per deposition area. **A.** Boxplot representing DNA quantity (ng) obtained per fingerprint area (cm2) with outliers. **B.** Bar graph representing DNA (ng) obtained per fingerprint area (cm2) without outliers. DH and from DNH from six donors are shown. Each series represent the five cumulative swabbing process. Error bars represent the standard error. No statistically significant results were detected between hands ($p > 0.05$).

and soap and allowed fingertips to naturally "recharge" for 10 min. A gentle finger tapping was applied to homogenize skin/sweat compounds at deposition. Each volunteer provided a total of 5 sets of samples in duplicate from each hand (dominant = DH and non-dominant = NDH) on a glass surface, previously sterilized. Latent fingerprints from DH and NDH were deposited and combined in the following fashion: DH 1 / NDH 1 = 1 fingerprint; DH 2 / NDH 2 = 2 fingerprints; DH 3 / NDH 3 = 3 fingerprints; DH 4 / NDH 4 = 4 fingerprints; and DH 5 / NDH 5 = 5 fingerprints, placed next to each other for pooling. Contrarily to the most modern technique of double flocked swabs [4], DNA was collected with 10 μL dd water wet cotton swab per sample combination.

DNA extraction from fingerprints, was performed with the Qiagen® QIAamp® DNA Mini Kit. DNA was quantified with the Human Quantifiler® kit using Applied Biosystems 7500 Real Time PCR System. DNA concentration values were normalized per fingerprint surface extension as ng/cm^2. Sample sets were amplified with AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit on a 9700 GeneAmp PCR System, first by following the manufacturers protocols (Fig. 2A) [5] and after by increasing the number of denaturing, annealing and extending stage cycles (Fig. 2B). The customized protocol included: single wet swabbing for collection and 32 cycles for PCR amplification. Capillary electrophoresis and STRs analysis by GeneMapper ID 3.2 software (AppliedBiosystems) was performed.

Statistical analyses were performed (Student t-test and two-way ANOVA) with R studio software [6]. ANOVA was followed by Tukey HSD post hoc comparisons. In both cases, an alpha value of 0.5 was set as statistical significance.

3. Results and discussion

Each of the two sets of fingerprints, DH and NDH (ng/cm^2), contained samples from five cumulative swabbing processes: DH1 to DH5 and NDH1 to NDH5. Three of sixty depositions, belonging to two donors' DH, were detected as outliers by R-studio software [6]. These differed greatly in terms of DNA titer, possibly due to operator's contamination, and were removed from further statistical analyses (Fig. 1A). No differences in DNA titers were detected ($p > 0.55$) between sexes and age groups.

For DH sets, regardless of biological sex and age, DNA recovery slightly increased up to three pooled depositions ($p > 0.5$) (from 0.015 ng/cm^2 to 0.021 ng/cm^2); however, when combining four and five fingerprints, a decrease in DNA quantity (0.05 ng/ cm^2) (Fig. 1B) and STR quality (Fig. 2A/B) was noted ($p > 0.5$). For the NDH, the yield increased gradually across finger combinations but was not statistically

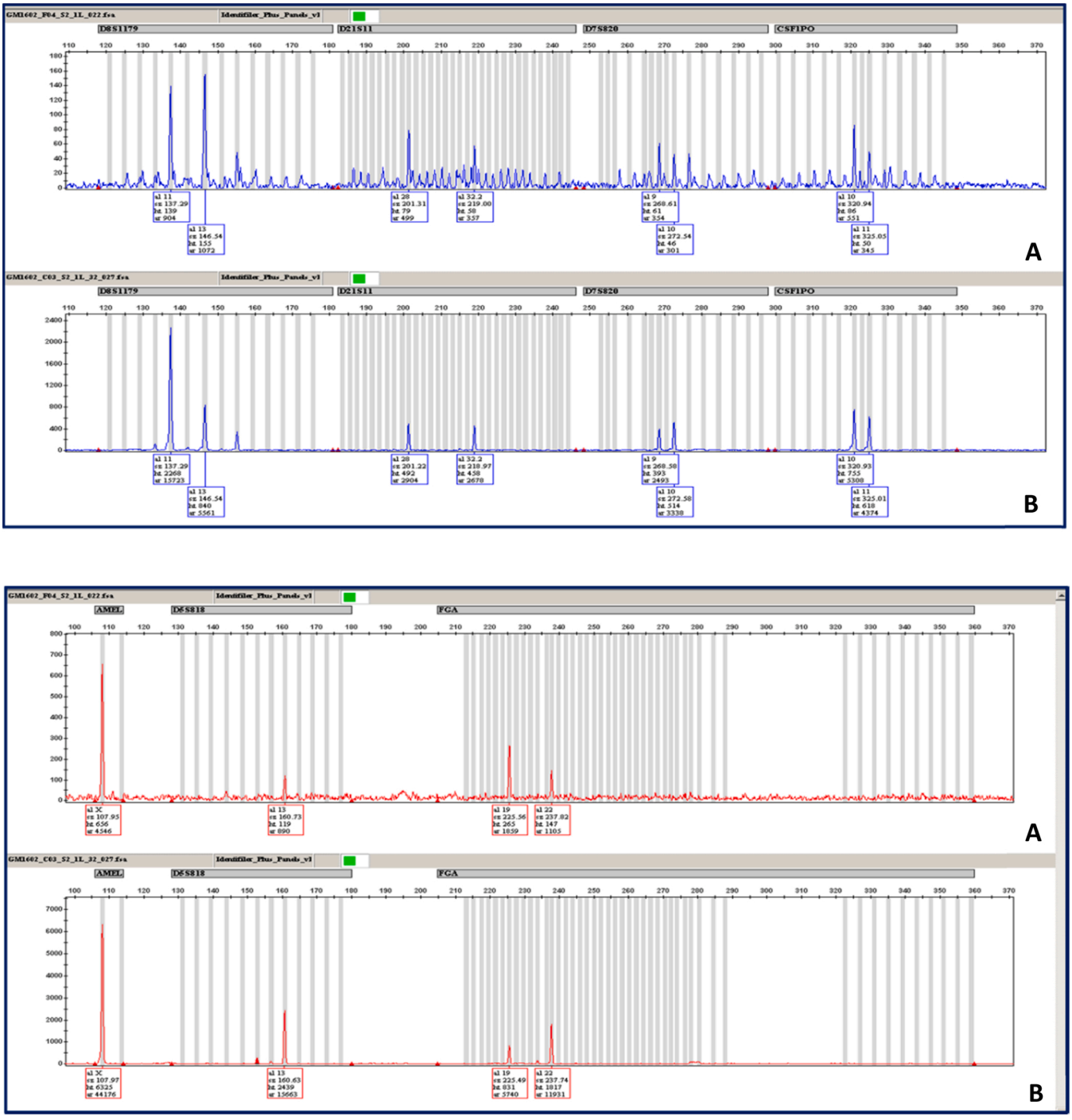

Fig. 2. Examples of electropherograms obtained from 0.002 ng/μL from NDH1 female donor. The sample was amplified by AmpFLSTR™ Identifiler™ Plus kit by manufacturer's protocol (**A**) and by our optimized customized protocol (**B**). STR profiles were improved in peaks' height and quality. Note: the artefact in D8S1179 locus.

significant (from 0.011 ng/cm^2 to 0.016 ng/cm^2) ($p > 0.5$) (Fig. 1B). Interestingly, NDH 4 and NDH 5 increased DNA recovery unlike DH counterparts (Fig. 1B). The difference between DH and NDH, as well as the effect of fingerprint combinations (Fig. 1B), revealed no significant interactions between DH/NDH and DNA yields ($p > 0.5$) with no finger combination effects ($p > 0.5$). The average DNA quantity obtained across all DH and NDH fingerprint area was the same (Fig. 1B) (0.070 ng/cm^2 DH and 0.067 ng/cm^2 NDH).

It is possible that with DH, the swab became saturated with fingerprint compounds and failed to collect more cellular material from the third fingerprint combination onward. For NDH, there could be less starting cellular material and therefore the swab did not saturate for the samples analyzed.

4. Conclusions

The authors have described a customized protocol for quantifying and normalizing DNA titers per cm^2 of fingerprint surface. This novel approach may provide a minimum required fingerprint area extension to obtain useful STR profiles. No significant differences in DNA quantity

were revealed between sex and age groups. DNA recovery was slightly improved up to three pooled fingerprints for DH and five for NDH, although not statistically significant. DH and NDH height peak electropherogram signals were enhanced with the modified protocol, producing STR profiles cleared of noise and artefacts.

Ethics approval and consent to participate

All participants accepted and signed the informed consent to their participation to the study that was previously approved by the Ethic Committees at the University of Barcelona (IRB-0.0003099).

Conflict of interest statement

None.

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