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Research Article

Optimization of a DNA extraction protocol from fingerprints for the analysis of nuclear STR and mitochondrial DNA genetic profiles

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Abstract

Most of the cells found in fingerprints belong to the cornified barrier of the epidermis (stratum corneum), lack nuclei and cytoplasmic organelles, and are filled with keratin. Nuclear and mitochondrial DNA is degraded and embedded in the keratin mesh, a very resistant protein that is difficult to remove during DNA extraction. In this work, we studied the possible negative effect of keratin on Polymerase Chain Reaction (PCR) reactions and the influence of keratinase and proteinase K on the extraction of DNA from fingerprints. The role of glycogen in the DNA yield during the precipitation step and the importance of washing the obtained DNA with 70% ethanol were also studied. DNA was extracted from 96 fingerprints corresponding to recent prints and stored for 0, 1, 5, and 18 months from six individuals. No differences were observed in the concentration of extracted DNA or in the number of nuclear Short Tandem Repeat (STR) alleles in the genetic profiles of fingerprints stored during different times. However, sex differences were observed in both the concentration of DNA obtained and the number of nuclear STR alleles detected, being lower in females than in males. In 80% of the fingerprints genetic profiles were obtained with at least half of the STR nuclear markers and, in 50% of the fingerprints genetic profiles were obtained with more than 90% of the markers, which would allow an unambiguous identification of the donor. In all fingerprints where mitochondrial DNA was analyzed, complete sequencing of the HV1 and HV2 regions was possible, which increases the accuracy of the results obtained. The optimized protocol allowed obtaining a complete STR nuclear genetic profile of a 20-year-old palmprint.

Key points

- The presence of keratin negatively influences PCR reactions.
- The addition of keratinase in latent fingerprint DNA extraction protocol improves the yield and quality of the DNA obtained.
- The use of glycogen for DNA precipitation and 70% ethanol for washing the precipitate influence the yield and quality of the DNA isolated from fingerprint.
- The DNA obtained is useful for STR marker profiling and HV1 and HV2 hypervariable regions of mitochondrial DNA analysis.
- The amount of DNA obtained and the number of STR markers detected from fingerprints depend on the sex of the individual, but not on the time elapsed. The optimized protocol is efficient in analysis of a 20-year-old sample.

Keywords: keratinase; latent fingerprint; STR analysis; HV1 mtDNA analysis; HV2 mtDNA analysis; forensic DNA typing

Introduction

The extraordinary technological development of the last decades has provided the scientific community with tools that allow the analysis of minimal amounts of DNA. However, the possibility of obtaining complete genetic profiles from biological samples, which can be used in comparison with reference samples for the resolution of forensic cases, depends fundamentally on the quality of the DNA obtained rather than the quantity. A quality DNA is one that is not broken or degraded and is free of contamination from proteins present in the cell and from chemical compounds used during extraction.

The biological samples found in the different scenarios are often highly degraded because of the time elapsed since they were deposited. The environmental conditions of humidity, light, and temperature in which they were found are responsible for the breakdown or degradation of the nucleic acids in these samples [1]. Fabric, glass, metal, plastics, wood, or paper does not have the same influence on the samples processing,

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because of their different adsorption capacity and the different chemicals with which they can be treated. Another critical factor is the cell type from which to extract DNA. Blood, semen, saliva, or anagen complete hairs are possibly the best sources of quality DNA, although other types of tissues such as bones, teeth, and nails are also suitable for DNA analysis and are widely used in the identification of bodies or body parts through genetic analysis [2–5]. Specific commercial kits can be used for their extraction that takes into account the particular characteristics of these tissues and minimizes the presence of inhibitors of the PCR reaction. It is for all these reasons that, despite the enormous effort developed in recent years by crime laboratories, collected in a high number of scientific articles describing different protocols and methodologies for DNA analysis of different biological samples (see [1] for a review), it is not possible to have a general protocol for the treatment of forensic samples, because each of them is different.

Latent fingerprinting is one of the most secure biometric technologies and is considered legitimate proof of criminal evidence anywhere in the world, and it serves as a source of DNA for obtaining genetic profiles in cases where fingerprint analysis is inconclusive. The influence of the different methods used for the enhancement of latent fingerprints on the quality of the DNA obtained has been evaluated utilizing allele loss analysis in genetic profiles [6–10]. Also, the amount of DNA in relation to the pressure exerted on deposition [11], variations between shedder categories and individuals [12], and different extraction methods organic, or with silica gel columns [1, 13], have been evaluated. However, in the contributions reviewed, we have not found any reference to the possible influence of keratin present in the cells of the fingerprints, on the DNA extraction process. Most of these cells belong to the cornified barrier of the epidermis (stratum corneum) efficiently protect us from the entry of external biological material [14]. However, they are dead cells lacking nuclei that progressively degrade as they migrate towards the surface of the epidermis through a mechanism similar to apoptosis called cornification [15, 16]. This means that the DNA of this cell type is already degraded. The cytoplasm of keratinocytes also undergoes modifications: the cytoplasmic organelles disappear, and the entire space is occupied by keratin, a very resistant protein that is very difficult to eliminate and, therefore, a possible contaminant of DNA isolated from prints. On the other hand, a high expression of DNases [14] has been found in the cells of the stratum corneum, compatible with the protective function of the epidermis against genetic agents coming from outside and which may act during the lysis of the cells in the process of DNA extraction from the fingerprints.

DNA extracted from a latent fingerprint contains nuclear and mitochondrial DNA (mtDNA), which could be used in forensic identification. Both types of DNA have advantages and disadvantages. Thus, in nuclear DNA, highly variable STR markers can be studied that allow virtually unambiguous identification of any individual. In contrast, in mtDNA, the variability found is much lower and is mainly located in the hypervariable regions HV1 and HV2 of the D-loop [17]. However, the number of molecules per cell of mtDNA is thousands. Thus, molecular markers from degraded samples are more likely to be obtained when analyzing mtDNA.

This work aims to establish a specific protocol for the extraction of latent fingerprint DNA, taking into account the intrinsic characteristics of keratinocytes to assess the quality of the isolated DNA and determine the minimum amount of fingerprint DNA required to obtain a complete genetic profile of nuclear STR markers, and the analyses of the hypervariable regions HV1 and HV2 of the mtDNA.

Materials and methods

Deposition of fingerprints

The fingerprints of three females (F1–F3) and three males (M1–M3) were placed on UV-sterilized glass slides for 20 min, under maximum pressure for fingerprint deposition, and stored at room temperature and in the dark for 0, 1, 5, and 18 months (96 fingerprints: four per individual, and time) until processing.

Swabbing fingerprinting

4N6 FLOQ Swabs (COPAN, Murrieta, CA, USA) soaked with 30 μL of 2% sodium dodecyl sulphate (SDS) were used for the swabbing.

DNA control extraction

DNA extracted from the buccal mucosa of the six individuals was used as control DNA. For this purpose, the Gentra Puregene Buccal Cell Core Kit A (QIAGEN, Hilden, Germany) was used following the manufacturer's protocol.

Quantification of DNA

Once the DNA was extracted, a first measurement was performed on the Thermo Scientific™ NanoDrop 2000 spectrophotometer (Waltham, MA, USA). Accurate quantification was performed with the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems; Thermo Scientific™) following the manufacturer's protocol, optimizing the reaction volume from 23 to 10 μL. The equipment used was the 7500 Fast Real-Time PCR System (Applied Biosystems).

PCRs for keratin inhibition tests

Fragments of the prothrombin (*Pro*) and T-plasminogen activator (*Tpa*) genes were chosen for 25 μL of PCR amplification with specific primers and amplification programmes (Table 1) from DNA control (50 ng), dead epithelial cells, and DNA control-dead epithelial cells mixtures as is described in Results and discussion section.

Nuclear genetic profiles

The AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit (Applied Biosystems) was used to amplify of STR markers. The fragments generated were separated by capillary electrophoresis using the 3130*xl* Genetic Analyzer (Applied Biosystems; Thermo Scientific™), and Genetic profiles were obtained with GeneMapper™ ID Software v3.2.1 (Applied Biosystems; Thermo Scientific™).

Analysis of mtDNA

The HV1 and HV2 hypervariable regions of the mitochondrial genome were amplified using the primers validated by Lee et al. [18]. Table 2 shows the sequences of the primers used for PCR and the amplification programme. The efficiency of PCR amplification was tested by separating fragments on 1%–1.5% agarose gels in TAE 1× (Tris-acetate-EDTA) or SB 1× (Sodium Boric Acid) buffer. Five microliter of the PCR reactions was purified by ExoSAP-ITM™ (Affymetrix; Thermo Scientific™) for subsequent sequencing by the Sanger method with the BigDye™ Terminator v3.1 kit (Applied Biosystems; Thermo Scientific™) and was separated by

Table 1. Primers and PCR programmes for the *Tpa* and *Pro* genes.

Gene	Primers	Number of cycles 30/40
Tpa	F: 5'GTAAGAGTTCCGTAACAGGACAGCT3' R: 5'CCCCACCCTAGGAGAACTTCTCTTT3'	1′ at 94°C, 30″ at 60°C, 30″ at 70°C
Pro	F: 5'CATGTGTTCCGCCTGAAGAAGTGG3' R: 5'ATAGCACTGGGAGCATTGAAGCT3'	30″ at 94°C, 30″ at 52°C, 45″ at 72°C

Table 2. Primers and PCR programme for the specific amplification of the HV1 and HV2 mtDNA regions. The primers in red were used for amplification of the complete HV1 and HV2 regions. All of them were used in the Sanger sequencing reactions.

Mitochondrial region	Primers	35 cycles
HV1	F15989: CCC AAA GCT AAG ATT CTA AT R16153: CAG GTG GTC AAG TAT TTA TGG F16097: TAC ATT ACT GCC AGC CAC CA R16233: TGA TAG TTG AAG GTT GAT TGC TGT F16159: CAT AAA AAC CCA ATC CAC AT R16304: ACT GTT AAG GGT GGG TAG GT F16247: ACT CCA AAG CCA CCC CTC A R16410: GAG GAT GGT GGT CAA GGG AC	20″ at 94°C 20″ at 56°C 30″ at 70°C
HV2	F015: CAC CCT ATT AAC CAC TCA CG R187: CGC CTG TAA TAT TGA ACG TA F120: CGC AGT ATC TGT CTT TGA TTC C R285: GTT ATG ATG TCT GTG TGG AA F220: TGC TTG TAG GAC ATA ATA AT R389: CTG GTT AGG CTG GTG TTA GG	

capillary electrophoresis using a 3130*xl* Genetic Analyzer (Applied Biosystems; Thermo Scientific™).

Statistical analysis

ANOVA, Fisher's least significant difference (LSD) test, and Pearson correlation were performed using StatGraphics plus software v.5.1 (Warrenton, VA, USA).

Results and discussion

Determination of the influence of keratin on PCR amplification of DNA from fingerprints

Different inhibitory molecules can negatively interfere with PCR reactions [19]. In stratum corneum cells, keratin is postulated as a possible contaminant of extracted DNA from fingerprints whose inhibitory role should be evaluated. For this purpose, PCR amplification assays were performed using aqueous suspensions of skin scrapings containing dead epidermal cells with a high amount of keratin: 100 μg/μL (A), 10 μg/μL (B), 1 μg/μL (C), and 0.1 μg/μL (D), respectively. The dead epithelial cells used were not subjected to any DNA extraction process in order to ensure the presence of keratin. Two microliter of each of the above suspensions were used for PCR amplification of fragments of the *Tpa* gene with two alleles (100 bp and 400 bp) (Figure 1A) and the prothrombin gene (147 bp) (Figure 1B). No amplification occurred in the epithelial cell suspensions (Lanes A to D).

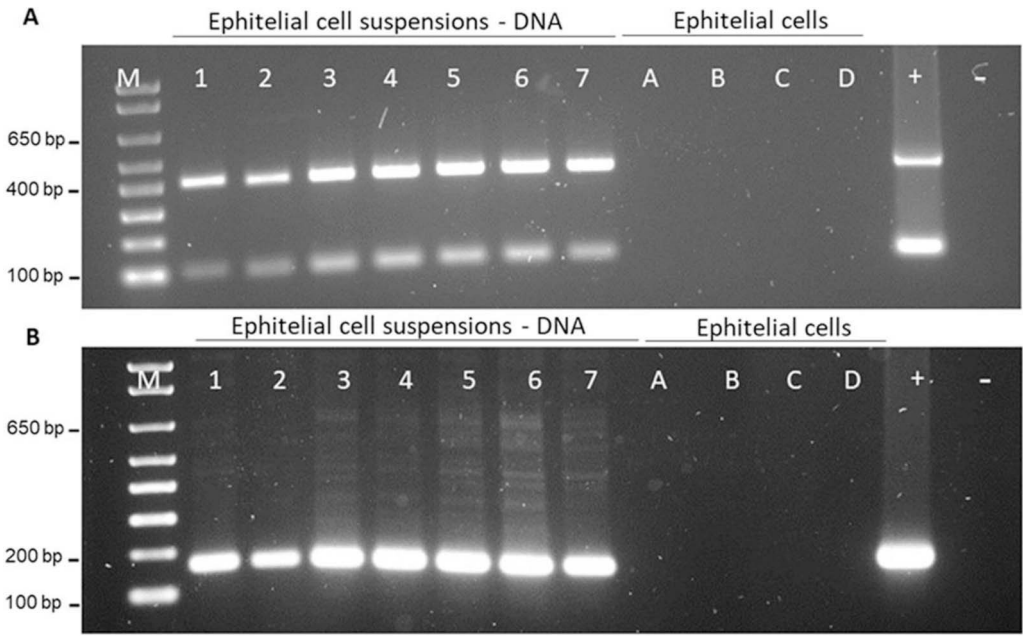

Figure 1 PCRs electrophoresis result (30 cycles) of the *Tpa* gene (A) and the *Pro* gene (B). M: molecular marker. 1–7: dead epithelial cell suspensions—control DNA (50 ng) mixtures. A–D: dead epithelial cell suspensions. +: positive control. −: negative control.

On the other hand, to discriminate whether the possible non-amplification was because of keratin or the low quantity and quality of DNA, it was decided to make mixtures of suitable quality control DNA (50 ng), and dead epithelial cells in the proportions showed in Table 3. Two microliter of each of the above mixtures were included in the PCR amplification assays for both genes (Figure 1). In the epithelial cell-DNA mixture (Lanes 1 to 7), fragments of both genes were amplified. However, the amplified bands were less intense in the mixtures with higher concentrations of dead epithelial cells (Lanes 1 and 2). Since the control DNA concentration in all solutions is the same, the lower amplification may be because of keratin exerting an inhibitory effect.

To determine whether the non-amplification in the case of using only dead epithelial cells was because of a very low DNA concentration, further PCRs were performed by increasing the number of cycles to 40. Figure 2 shows the PCR amplification with the *Tpa* gene primers. It is important to note that the dead epithelial cells were from an individual homozygous for the 100 bp fragment.

As the number of amplification cycles increased, amplification was observed in Solutions B and C. No amplification occurred in Solution A with a higher concentration of dead epithelial cells (100 μg/μL), keratin, and presumably of DNA. In Solutions B (10 μg/μL) and C (1 μg/μL), it seems that there is enough DNA for amplification to occur, and the amount of keratin is not sufficient to inhibit the reaction. In Solution D with the lowest concentration of cells (0.1 μg/μL), amplification did not occur either. In this case, the cause must be the insufficient amount of quality DNA for amplification.

It should be noted that in the presence of keratin, the smaller 100 bp fragment of the *Tp*a gene amplifies worse than the 400 bp fragment in comparison to the control DNA (Figures 1 and 2). This may be because smaller degraded DNA fragments may be trapped in the keratin mesh, making them more inaccessible to *Taq* polymerase in the PCR reaction. Whereas larger fragments, which will also be trapped in the keratin mesh, could expose a larger part of their sequence and be recognized more efficiently by *Taq* polymerase.

Table 3. Weight ratio between dead epithelial cells and control DNA (1 = 50 ng) for determination of the influence of keratin in the PCR reactions.

Suspension	Epidermal cells:control DNA
1	1 000:1
2	500:1
3	100:1
4	50:1
5	10:1
6	5:1
7	1:1

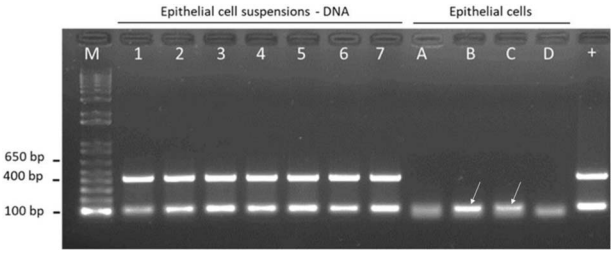

Figure 2 Electrophoresis results of PCRs (40 cycles) of the *Tpa* gene. M: molecular marker. 1–7: epithelial cell suspensions—control DNA. A–D: epithelial cell suspensions. +: positive control. Arrows point to the fragment amplificated of 100 bp.

Development of a DNA fingerprinting extraction protocol

Since there are no specific kits for DNA extraction from fingerprints that take into account the particular characteristics of these samples, different DNA extraction kits varying methods of purification (columns, magnetic beads, or direct precipitation) were tested in the laboratory: NZY Tissue gDNA Isolation kit (Nzytech), PrepFiler™ Forensic DNA Extraction Kit (Applied Biosystems), and Gentra Puregene Buccal Cell Core Kit A (QIAGEN). Ten fingerprints were used with the three extraction kits (data not shown). Only with the latter, with the precipitation method, was a measurable amount of DNA obtained with NanoDrop, so it was chosen to optimize the DNA extraction protocol.

Considering the particular characteristics of enucleated cells of the stratum corneum of the skin, in which DNA is digested by nucleases [14], it was thought that the addition of proteinase K to the lysis buffer would prevent these enzymes from continuing to act, causing complete degradation of the DNA. On the other hand, as deduced from the previous results, keratin may inhibit PCR when the quantity and quality of DNA is low. Therefore, the use of keratinase in the DNA extraction process was also tested. Given these two premises, three modifications to the basic protocol of the Gentra Puregene Buccal Cell Core Kit A (QIAGEN) were tested, consisting of the addition of keratinase and/or proteinase K to the lysis buffer and different incubation times (Table 4).

A flowchart of the general procedure of the selected kit is shown in Figure 3. Three fingerprints were tested per protocol, and the method chosen to detach the cells from the slide was swabbing with 2% SDS [20].

To estimate the quantity and purity of the DNA extracted with the different protocols, 1 μL of 12 fingerprints was measured in a NanoDrop spectrophotometer (Table 5). It should be taken into account that because of the small amount of DNA obtained and the possible presence of other molecules from the extraction kit solutions, the values are estimates of the actual DNA concentration, although they allow comparison between samples. In general, all 260/280 absorbance ratios are higher than 2 (data not shown), confirming that the concentration measurement obtained with the NanoDrop is not exclusively because of DNA but other compounds.

An ANOVA was performed for the DNA concentration of each fingerprint, taking the extraction protocol used as an independent factor. The result of the ANOVA was significant ($P = 0.003$), and an LSD test was performed to know the differences between protocols (Figure 4).

The highest DNA concentration was obtained from the fingerprints extracted with Method B (Table 5), which includes the addition of keratinase with 30 min of incubation at 50°C. Protocol C including only the addition of proteinase K did not improve the yield of extracted DNA compared with the standard protocol (Protocol A). In Protocol D, adding of keratinase for an incubation time of 15 min improved the extraction yield even though this protocol included a subsequent incubation with proteinase K for 15 min. These

Table 4. Modifications (B–D) to the basic protocol (A) of the Gentra Puregene Buccal Cell Core Kit A (QIAGEN).

Protocol	Keratinase	Temperature/incubation time	Proteinase K	Temperature/incubation time
A	-	-	-	-
B	1 unit	50°C/30 min	-	-
C	-	-	20 μg	55°C/30 min
D	1 unit	50°C/15 min	20 μg	55°C/15 min

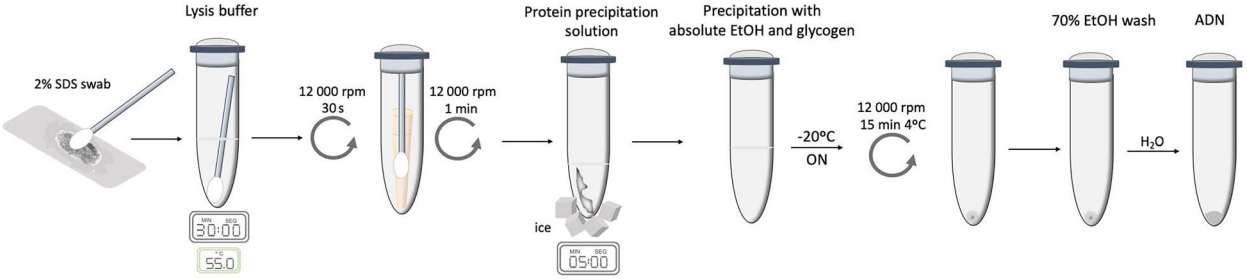

Figure 3 Flowchart of the general DNA extraction procedure with the Gentra Puregene Buccal Cell Core Kit A (QIAGEN).

Table 5. DNA concentrations (ng/μL) of the 12 fingerprints (three per protocol) extracted with the protocols: A: basic protocol; B: protocol with keratinase; C: protocol with proteinase K; D: protocol with keratinase and proteinase K for 30 min.

Protocol	Concentration (ng/μL) FP1	Concentration (ng/μL) FP2	Concentration (ng/μL) FP3
A	11.8	9.0	8.5
B	13.0	12.8	13.2
C	9.3	5.9	5.9
D	11.8	11.2	11.5

data confirm the usefulness of adding keratinase during the fingerprint DNA extraction process, whereas the addition of proteinase K seems to reduce the DNA harvest.

To analyze the possible influence of the four protocols on PCR amplification, DNA from all samples was used to amplify the *Tpa* gene fragments. The results showed a better yield of the B protocol with the highest intensity of the amplified fragments, as expected, depending on the concentration of DNA extracted. Figure 5 shows an agarose gel with the results of PCR amplification of the *Pro* gene with two DNA samples per protocol.

For many years, forensic laboratories have successfully used DNA extraction protocols in samples with high keratin content (nails, hair, etc.), using proteinase K and DTT. In our work we did not used DTT, as keratinase has potent multifunctional enzymatic activities that attack disulphide and peptide bonds, thus effectively depolymerising keratin. Based on our results, we can propose the usefulness of keratinase in the protocols commonly used in forensic laboratories for DNA extraction from samples with high keratin content.

Analysis of DNA precipitation with glycogen and washing of the precipitate with 70% ethanol to obtain genetic profiles

Before obtaining the genetic profiles of the DNA extracted from the fingerprints, the possible influence of the addition of glycogen on the precipitation process and the possibility that part of the DNA might be lost during the washing with 70% ethanol was raised. This is because very small DNA fragments

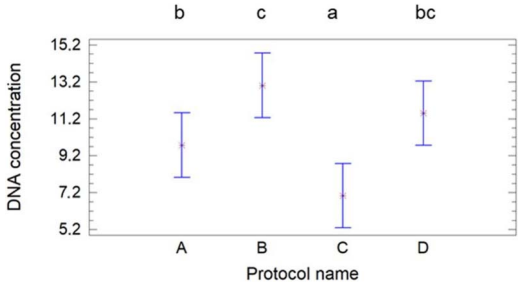

Figure 4 Graphic representation of the mean DNA concentration and least significant difference (LSD) test obtained from the four extraction protocols tested (A–D). Protocols with the same letter (lower case letters) are not statistically different ($P < 0.05$).

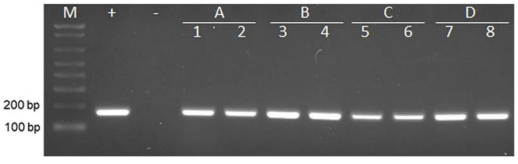

Figure 5 Electrophoresis results of PCRs (35 cycles) for *Pro* gene. M: molecular marker. 1–8: DNA from fingerprints tested with different protocols: Protocol A (1–2), Protocol B (3–4), Protocol C (5–6), Protocol D (7–8). +: positive control. −: negative control.

may have difficulty precipitating and/or dissolving with the salts in the aqueous part of 70% ethanol.

DNA from eight fingerprints was extracted using Protocol B up to the protein precipitation step. Next, all super-

natants were mixed in the same centrifuge tube and then separated into four aliquots to finish the extraction with the modifications indicated in Table 6 about the use of glycogen and 70% ethanol. DNA from all four aliquots was resuspended with the same volume of water, regardless of whether glycogen and/or 70% ethanol had been previously added to the sample. To evaluate the effectiveness of each option, genetic profiles were obtained from the DNA collected. Figure 6 shows a profile example of STRs labelled with the 6-FAM™ fluorochrome.

The results confirm the usefulness of glycogen in the precipitation process of samples with little DNA to avoid the loss of genetic material after discarding absolute alcohol once precipitated. On the other hand, a higher amount of DNA seems to be obtained when not washing with 70% ethanol (G in Figure 6), as shown by the appearance of higher peaks in the larger STR markers (>200 bp). However, in the quantification assays of these same samples using the Quantifiler™ Human

Table 6. Glycogen and 70% ethanol utilization assays in the precipitation and washing of DNA salts, respectively. +: glycogen or 70% ethanol added. −: not addition of glycogen or ethanol.

Protocol	Glycogen precipitation	70% ethanol washing
G70	+	+
G	+	−
70	−	+
X	−	−

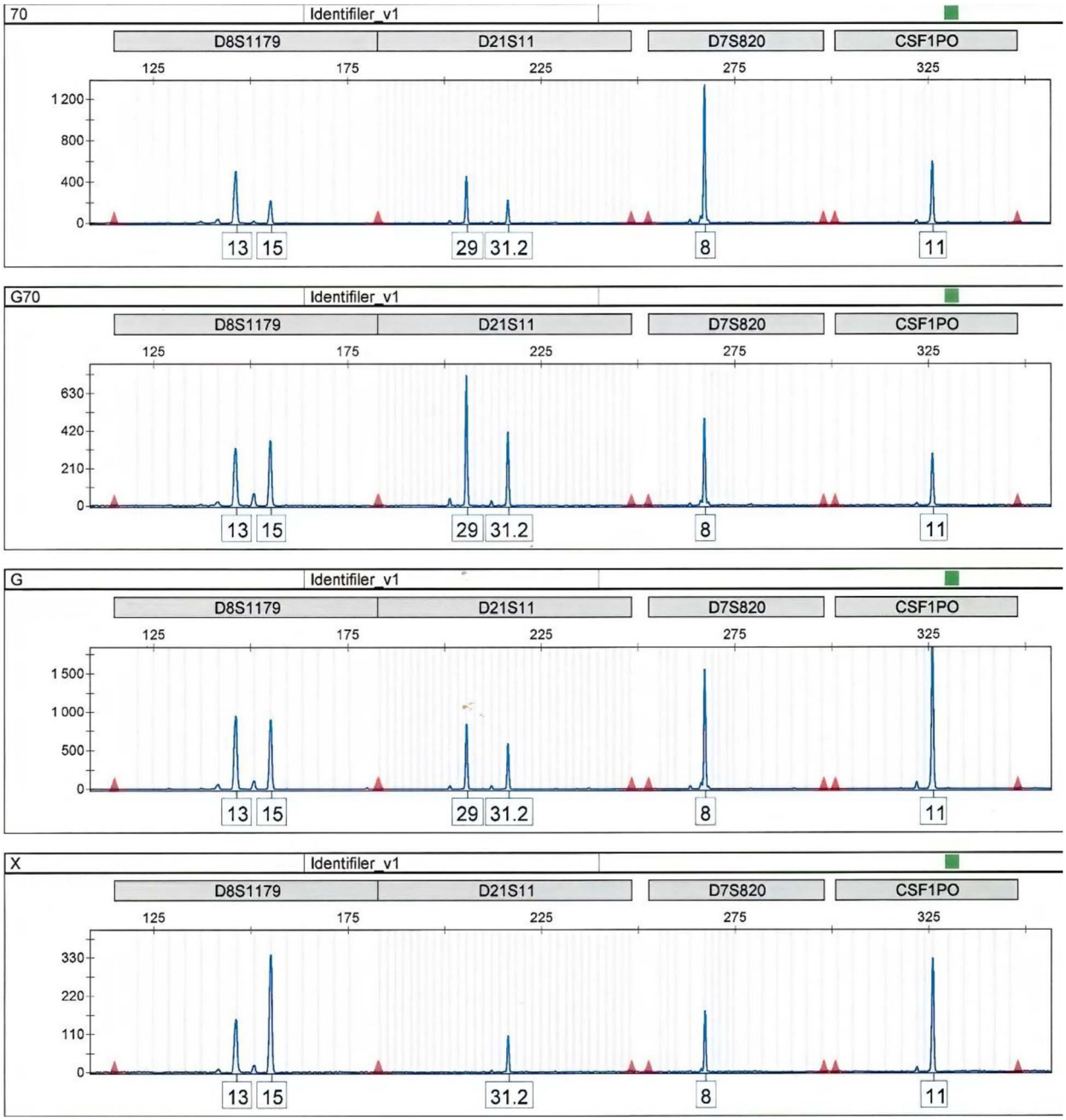

Figure 6 STR markers (6-FAM™ fluorochrome) from electropherograms of precipitate (glycogen) and salt wash tests (70% ethanol). (70) Without glycogen and with 70% ethanol; (G70) with glycogen and with 70% ethanol; (G) with glycogen and without 70% ethanol; (X) without glycogen and 70% ethanol.

DNA Quantification kit (next section), a lack of amplification of the Internal PCR Control (IPC) appeared in the samples that had not been washed with 70% ethanol. The IPC is a synthetic DNA sequence included in the kit whose non-amplification determines the presence of inhibitors in the PCR reaction. This led us to think that, although 70% of EtOH washing loses genetic material, it also serves to purify salts or other molecules that could inhibit the quantification reactions. However, not washing with 70% EtOH does not affect the obtaining of genetic profiles with the AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit, and the 70% EtOH washing can be eliminated in the case of samples that do not require an accurate quantification. Possibly, the PCR buffer in I AmpFℓSTR™ Identifiler™ Plus contains components that are able to neutralize some types of inhibitors, whereas this is not the case for the Quantifiler™ PCR buffer.

Quantification of the concentration of DNA extracted from fingerprints

Accurate quantification of the extracted DNA was performed with the Quantifiler™ Human DNA Quantification Kit using a 62 b probe homologous to the *Telomerase Reverse Transcriptase* (*hTERT*) gene, which contains a fluorochrome that is released when the Taq polymerase enzyme reaches it in a qPCR reaction. Although quantification is very accurate for a good quality DNA because the probe can bind to a very small fragment of DNA, in the case of DNA extracted from fingerprints, it is randomly degraded. Therefore, the complementary strand to the probe may be broken at the binding site and underestimate DNA concentration. To test the repeatability of the quantification obtained from fingerprints with the Quantifiler™ Human DNA Quantification Kit, two DNA measurements of 24 fingerprints were performed (Table 7). The correlation between both measures was 0.99 ($P < 0.001$), which would validate the use of the Quantifiler™ Human DNA Quantification Kit as a method to estimate the DNA concentration extracted from fingerprints. From these results, the Quantifiler Human DNA Quantification kit was used to estimate the DNA concentration of all the fingerprints analyzed in this work, yielding DNA extraction ranging from 0.011 to 5.112 ng/μL (Table 8). Interestingly, a very high amount of DNA was obtained in the 0-M2-3, 1-M2-3, 18M3-4 fingerprints. It should be noted that, although it has been assumed for years that the DNA found in fingerprints comes from shed external skin cells, it is possible that such DNA comes not only from keratinocytes shed from the surface layer of the finger, but also from nucleated epithelial cells, other parts of the body, or other fluids, as well as deposits of free DNA from endogenous cells [21].

Obtaining and studying the genetic profiles from latent fingerprints

Since 93.75% (90/96) of the DNA obtained from the fingerprints (Table 8) had a concentration below 1 ng/μL, which is the concentration recommended by the AmpFℓSTR™ Identifiler™ Plus PCR amplification kit, we decided to use 1 μL of the DNA extracted from each of the fingerprints regardless of their concentration obtained by the Quantifiler Human DNA Quantification Kit to estimate the minimum amount of DNA needed to obtain a complete genetic profile. It should be noted that, in the samples where the concentration was close to 1 ng/μL, highly saturated profiles were obtained (Figure 7A), where it was not possible to identify the alleles of the largest STR markers in each reaction. This may be because of the fact that when DNA concentrations in the PCR reaction are high, amplification of small fragments is favoured, exhausting the reaction components for amplification of larger markers. In these cases, the amplification was repeated with a dilution of one-fifth of the initial sample concentration, obtaining complete profiles (Figure 7B). Following the optimized protocol, complete genetic profiles with all the alleles corresponding to the individual according to their control profile were obtained from DNA concentrations of 0.179 ng/μL (Table 8).

Table 7. Quantifiler™ results (ng/μL) for two replicates of 24 samples. F: female; M: male.

FP	Measure 1 (ng/μL)	Measure 2 (ng/μL)
F1.1	0.09	0.13
F1.2	0.60	0.60
F1.3	0.18	0.23
F1.4	0.53	0.35
M1.1	0.46	0.46
M1.2	0.24	0.24
M1.3	0.19	0.13
M1.4	0.25	0.28
F2.1	0.50	0.46
F2.2	0.63	0.60
F2.3	0.46	0.53
F2.4	0.44	0.38
M2.1	0.30	0.27
M2.2	0.05	0.09
M2.3	4.92	5.31
M2.4	0.88	0.81
F3.1	0.26	0.31
F3.2	0.17	0.13
F3.3	0.08	0.08
F3.4	0.65	0.68
M3.1	0.68	0.61
M3.2	0.14	0.11
M3.3	0.23	0.26
M3.4	0.06	0.06

Influence of fingerprint deposition time on genetic profiles

The results obtained by other authors regarding the degradation of DNA from fingerprints stored are contradictory. Some indicate a progressive degradation of DNA over time, with a decrease in the number of STRs, identified after 10 days [13], whereas Romano et al. [22] have managed to obtain partial DNA genetic profiles of 14-year-old fingerprints.

To study the influence of time on the quality of the extracted DNA and the genetic profiles obtained, evaluated by the number of STR alleles detected, fingerprints deposited at different times: recent (0), 1, 5, and 18 months were analyzed. Table 8 shows the results of the genetic profiles obtained from the fingerprints analyzed for each individual according to the time of fingerprint deposition.

An ANOVA was conducted for the number of STR alleles per fingerprint, taking the time elapsed between DNA deposition and DNA extraction as an independent factor. Data from fingerprints with a DNA concentration measured by the Quantifiler Human DNA Quantification Kit was <0.016 ng/μL (DNA concentration that the kit can reproducibly detect, according to the manufacturer) were

Table 8. Results of the DNA quantification by Quantifiler Kit, and the number of alleles presents of samples from different times: (0) recent; (1) 1 month; (5) 5 months; (18) 18 months. Sample names include: age (0–18), sex of individual (F or M), individual number (1–3), sample number (1–4).

Finger-print	DNA con-centration (ng/μL)	Num-ber of alleles	Fingerprint	DNA concentration (ng/μL)	Number of alleles	Fingerprint	DNA concentration (ng/μL)	Number of alleles	Fingerprint	DNA concentration (ng/μL)	Number of alleles
0-F1-1	0.068	30	1-F1-1	0.110	18	5-F1-1	0.087	31	18-F1-1	0.050	12
0-F1-2	0.056	12	1-F1-2	0.601	24	5-F1-2	0.013	6	18-F1-2	0.037	14
0-F1-3	0.018	21	1-F1-3	0.204	31	5-F1-3	0.011	0	18-F1-3	0.179	32
0-F1-4	0.151	27	1-F1-4	0.439	31	5-F1-4	0.013	5	18-F1-4	0.021	18
0-F2-1	1.257	32	1-F2-1	0.478	30	5-F2-1	0.734	32	18-F2-1	0.245	32
0-F2-2	0.215	25	1-F2-2	0.616	32	5-F2-2	0.085	31	18-F2-2	0.106	29
0-F2-3	0.053	12	1-F2-3	0.492	25	5-F2-3	0.128	28	18-F2-3	0.419	32
0-F2-4	0.054	16	1-F2-4	0.408	32	5-F2-4	0.088	28	18-F2-4	0.308	32
0-F3-1	0.043	10	1-F3-1	0.285	31	5-F3-1	0.018	10	18-F3-1	0.004	13
0-F3-2	0.048	16	1-F3-2	0.151	24	5-F3-2	0.099	17	18-F3-2	0.055	22
0-F3-3	0.041	8	1-F3-3	0.076	24	5-F3-3	0.000	24	18-F3-3	0.035	9
0-F3-4	0.017	8	1-F3-4	0.666	32	5-F3-4	0.000	0	18-F3-4	0.029	21
0-M1-1	0.252	28	1-M1-1	0.462	16	5-M1-1	0.923	32	18-M1-1	0.072	31
0-M1-2	0.641	29	1-M1-2	0.284	28	5-M1-2	0.000	0	18-M1-2	0.227	32
0-M1-3	0.263	28	1-M1-3	0.161	30	5-M1-3	1.027	30	18-M1-3	0.140	31
0-M1-4	0.128	26	1-M1-4	0.263	26	5-M1-4	1.325	31	18-M1-4	0.439	32
0-M2-1	0.199	32	1-M2-1	0.284	31	5-M2-1	0.234	30	18-M2-1	0.046	17
0-M2-2	0.389	29	1-M2-2	0.070	18	5-M2-2	0.198	23	18-M2-2	0.105	25
0-M2-3	2.207	32	1-M2-3	5.112	32	5-M2-3	0.003	20	18-M2-3	0.178	31
0-M2-4	0.000	30	1-M2-4	0.841	32	5-M2-4	0.257	32	18-M2-4	0.217	32
0-M3-1	0.134	29	1-M3-1	0.646	32	5-M3-1	0.087	18	18-M3-1	0.439	32
0-M3-2	0.028	25	1-M3-2	0.122	8	5-M3-2	0.116	29	18-M3-2	0.215	31
0-M3-3	0.183	30	1-M3-3	0.247	31	5-M3-3	0.324	32	18-M3-3	0.476	32
0-M3-4	0.345	32	1-M3-4	0.062	0	5-M3-4	0.076	21	18-M3-4	2.979	32

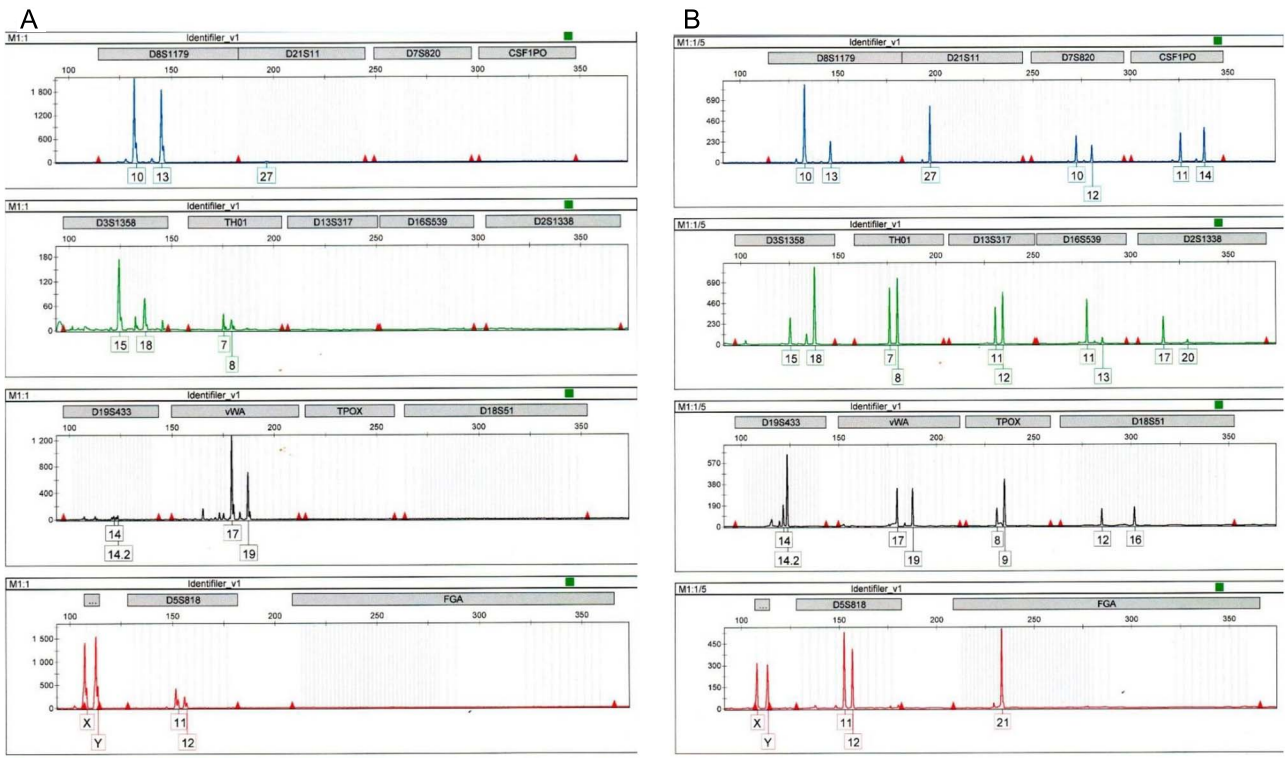

Figure 7 (A) Genetic profile of a sample with concentrations close to 1 ng/μL. (B) Genetic profile of the previous sample diluted 1/5.

excluded. The result of the ANOVA test was non-significant ($P = 0.52$). Therefore, according to our results the time of fingerprint deposition (0–18 months) does not influence the number of STR alleles obtained.

Influence of STR marker size for detection in fingerprint DNA

Table 9 shows the average allele size of the STR loci studied, and the number of times they have been detected in the set of

Table 9. The average allele size of the STR loci studied, and the number of times they have been detected in the set of latent prints studied.

Locus name	Alleles mean size of the locus	Number of alleles detected
D8S1179	139.28	148
D21S11	206.83	143
D7S820	271.2	126
CSF1PO	329	119
D3S1358	130	146
TH01	177	146
D13S317	226	137
D16S539	278	127
D2S1338	335.12	114
D19S433	121.67	153
vWA	179	154
TPOX	236	142
D18S51	297	120
AMEL	110	172
D5S818	151.4	148
FGA	234.33	131

genetic profiles of the latent fingerprints studied. The correlation between both parameters was −0.9318 ($P < 0.001$). The negative value of the correlation indicates that the larger the fragment size, the more alleles are lost or dropout (Figure 8). Our results agree with those obtained by other authors who indicate that the larger the size of the marker analyzed, the more likely it is that there is no amplification in samples with degraded DNA [23].

Influence of sex on DNA concentration and STR detection of the fingerprints

To determine if there are differences in the amount of DNA deposited and extracted in the fingerprints of females or males, an ANOVA was conducted comparing the DNA concentration of fingerprints according to sex. The result was statistically significant ($P = 0.03$). Thus, a mean concentration per fingerprint of 0.51 ng/μL was obtained for males, whereas for females, the mean concentration was 0.21 ng/μL. A similar study was carried out but took into account the number of alleles per fingerprint as a function of sex. The mean number of alleles per fingerprint in females and males was 23.1 and 27.4, respectively. The result of the ANOVA to compare the number of alleles per fingerprint between the sexes was significant ($P = 0.008$).

Our study shows that the sex of the donor influences both the concentration of DNA obtained from the fingerprints and the number of alleles detected in the genetic profile, which is consistent with previous studies by other researchers indicating that there is a more significant deposition of DNA by males than females [24–26]. However, our results contradict those obtained by Lowe et al. [27], who stated that detecting gender bias amongst the different donors was impossible. In a study by Phipps and Petricevic [28], the influence of sex was not taken into account, as it was not considered to be a relevant variable.

However, the number of individuals of each sex used in this study is small. To determine the influence of sex on the amount of DNA extracted from fingerprints, it would be necessary to increase the sample size and take into account other factors such as the size of the fingerprint, the amount of sweat excreted, etc.

Application of DNA optimized extraction protocol with old prints

To test if the optimized DNA extraction protocol allows DNA extraction from ancient fingerprints, the Physical Anthropology Laboratory of the University of Alcalá de Henares provided a palmprint of a girl deposited in 2002 (Figure 9). The use of the palmprint has been approved by the Committee on the Ethics of Animal Research and Experimentation at the University of Alcalá (code: CEI and EA: 2014/001/20141030). The palmprint were obtained using of the adhesive paper and graphite method described by Aase and Lyons [29].

The palmprint was swabbed with 2% SDS for DNA extraction using Protocol B described. DNA concentration of 0.49 ng/μL was obtained, and 1 μL was used to obtain the genetic profile. A complete genetic profile was observed that perfectly matched the markers of the control DNA profile (Figure 10). This demonstrates the usefulness of the optimized protocol with keratinase for obtaining genetic profiles from at least 20-year-old samples.

Analysis of mtDNA from fingerprints

In the cornification process of the stratum corneum, degradation of mitochondria and other cytoplasmic organelles also occurs. mtDNA is randomly fragmented like nuclear DNA.

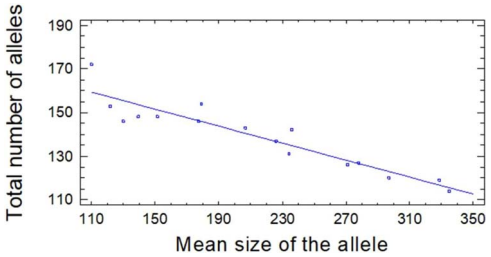

Figure 8 Linear regression line between the number of times the STR markers have been detected in the set of genetic profiles of the latent fingerprints studied (total number of alleles) and mean size of STR alleles.

Figure 9 Photograph of a girl's palmprint taken in 2002.

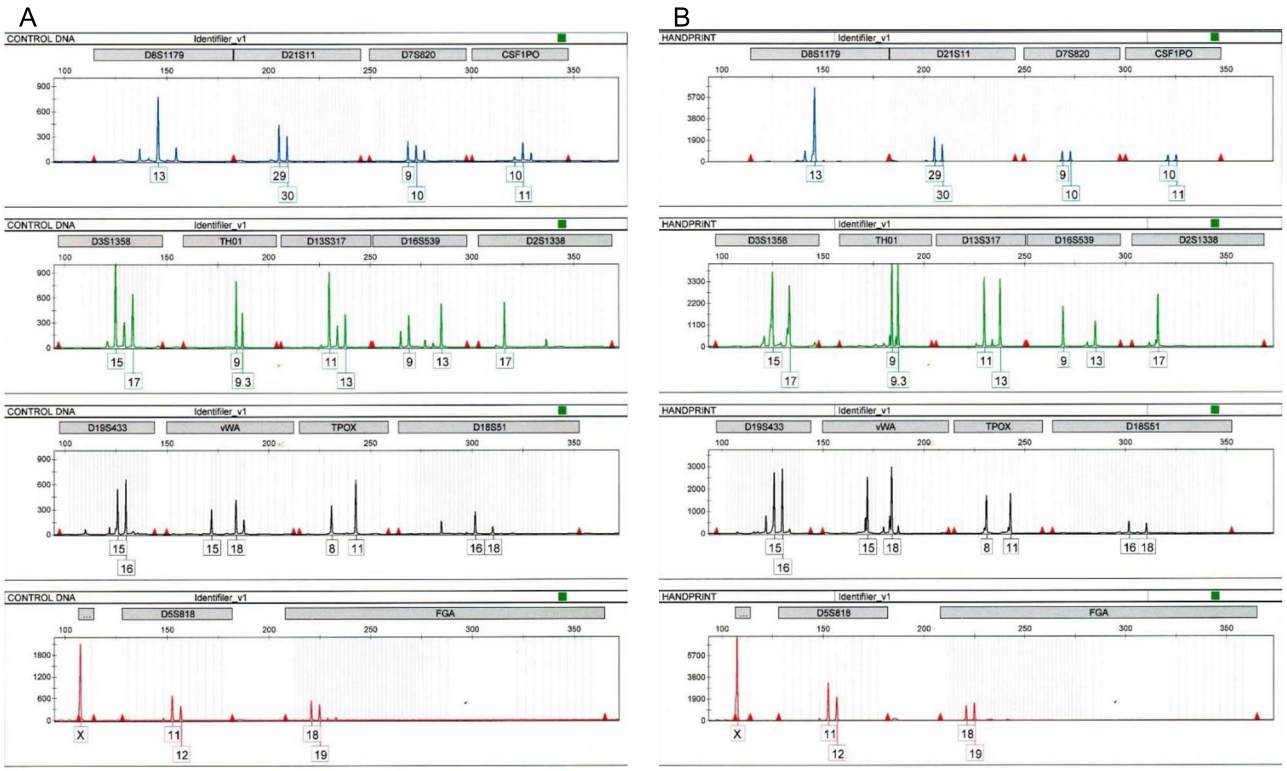

Figure 10 Electropherograms of the genetic profiles from the (A) palmprint (2002) and (B) DNA control. Each colour corresponds to labelling with different fluorochromes.

However, the higher number of molecules per cell of mtDNA provides several fragments that allow PCR amplification of specific regions from degraded samples [17].

In the present work, when DNA extracted from a fingerprint does not allow a complete genetic profile of nuclear STRs, the use of mtDNA was considered to expand the number of analyzable genetic markers to facilitate better identification of the individual from whom the sample was taken.

The hypervariable regions HV1 and HV2 of the mitochondrial genome of the DNA extracted from the 18-month-old fingerprints were chosen for this analysis for comparison with the control mtDNAs from the six individuals. For PCR amplification of both DNA strands of the HV1 and HV2 regions, primers designed by Lee et al. [18] for amplification of degraded DNA from bone were used, as indicated in the Materials and methods section. Prior to the analysis of the hypervariable regions of the latent prints mtDNA, the HV1 and HV2 regions of the control samples were sequenced by Sanger method. As only one amplification band was obtained with each primer combination, it was expected that the sequence could be obtained from the PCR product. The sequences obtained were compared with the mtDNA reference sequence from the MITOMAP database. The identification of the variants found was carried out with the bioinformatics tool MITOMASTER [30]. Table 10 shows the edition ranges of the HV1 and HV2 regions analyzed and the variants observed in each individual with respect to the reference human mtDNA sequence (rCRS).

All of them presented specific polymorphisms that allowed their individual identification. One microliter of the 24 samples was used to amplify HV1 and HV2 regions. The results of the amplifications are shown in Figure 11. Both regions were amplified in all samples, although with differences in the intensity of the bands obtained, which correlated with the concentration of nuclear DNA in each sample. The HV2 region was amplified more efficiently than the HV1 region, suggesting that the primers used for HV2 amplification perform better than those used for HV1 amplification and should be the primers of choice for critical samples with lower DNA concentration (Figure 11). In addition, in our work, we observed a greater variation in the HV2 region (Table 10). To

Table 10. Edition range analyzed and variants found in the HV1 and HV2 sequence in relation to the reference mtDNA sequence (rCRS) in the six mtDNAs of the individuals studied.

Edition range	HV1 (15 989–16 410)	HV2 (15–392)
M1	G16129A	A263G, T310C
M2		C315CC
M3	T16189C, C16252T, C16270T, G16319A	A73G, C150T, A200G, A263G, C309CCT, T310C
F1	T16304C	A93G, A263G, C315CC
F2	T16362C	C150T, T239C, A263G, T310C, T310TTC, T334d
F3	T16189C	A263G, T310C, C312T, G316C, T318C, T319G

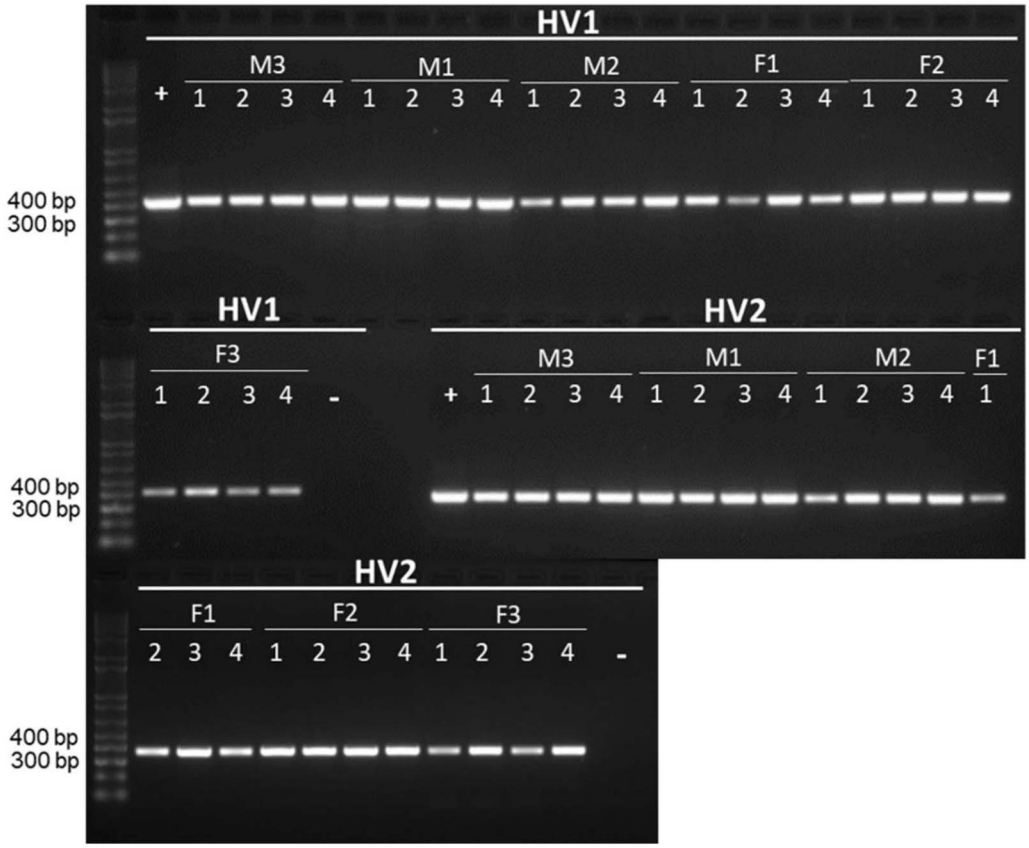

Figure 11 PCR electrophoresis result of HV1 and HV2 regions from 24 latent prints stored for 18 months. M: male (M1–M3). F: female (F1– F3). 1–4: fingerprints. +: positive control, −: negative control.

corroborate whether the amplified fragments of the HV1 and HV2 regions of the analyzed fingerprints corresponding to the individuals from whom the DNA was extracted, the sample with the lowest concentration of nuclear DNA from each of the participants was sequenced. In all cases, the sequences obtained from the fingerprints matched those of the control samples. It has been shown that with critical concentrations of 0.004 ng/μL of an 18-month-old fingerprint (Table 8) it is possible to get the complete HV1 and HV2 mtDNA sequences.

Conclusion

A protocol for DNA extraction from latent fingerprint DNA has been optimized, taking into account the characteristics of this biological material, such as its degradation and high keratin content. Adding of keratinase during the DNA extraction procedure with the Gentra Puregene Buccal Cell Core Kit Qiagen DNA kit (Protocol B), improved the yield of DNA isolated from a latent fingerprint. It is demonstrated that glycogen facilitates DNA precipitation while washing with 70% ethanol decreases the amount of DNA obtained.

In 80% of the fingerprints, genetic profiles were obtained with at least half of the STR nuclear markers and in 50% of the prints, genetic profiles were obtained with more than 90% of the markers, which would allow an unambiguous identification of the donor. In all fingerprints where mtDNA was analyzed, complete sequencing of the HV1 and HV2 regions was possible.

Sex differences have been observed in the amount of DNA extracted per fingerprint and in the number of STR markers detected, which are higher in males than in females. However, a larger number of individuals should be analyzed to determine the influence of sex on the amount of DNA obtained from fingerprints and to have better statistical support.

No differences were observed between fingerprints deposited from 0 to 18 months in the concentration of DNA extracted and in the number of molecular markers obtained. Furthermore, the proposed protocol demonstrates the possibility of getting complete genetic profiles from samples up to 20 years old.

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Authors' contributions

Yolanda Loarce and Juan M. González conceptualized the study. Yolanda Loarce, Pilar Rubio, Almudena Sánchez-Sanz, Ariadna Higuera, José A. Rodríguez-Pascual, Julián Blanco, Esperanza Gutiérrez-Redomero, and Juan M. González conducted the experiments and performed the data analysis. Pilar Rubio, Almudena Sánchez-Sanz, and Ariadna Higuera specifically carried out the genetic profile analyses. Yolanda Loarce and Juan M. González prepared the original draft of the manuscript. All authors contributed to the final text and approved it.

Compliance with ethical standards

The study's purpose and sample collection were approved by the Committee on the Ethics of Animal Research and Experimentation of the University of Alcalá (Code: CEI and EA: 2014/001/20141030). Participants in this study provided their written informed consent.

Disclosure statement

None declared.

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References

1. Lee SB, Shewale JG. DNA extraction methods in forensic analysis. In: Meyers RA, editor. Encyclopedia of analytical chemistry: applications, theory, and instrumentation. Chichester (NY): John Wiley & Sons, Ltd. 2017.
2. Guha P, Das A, Dutta S, et al. A rapid and efficient DNA extraction protocol from fresh and frozen human blood samples. J Clin Lab Anal. 2018;32:e22181.
3. Mullegama SV, Alberti MO, Au C, et al. Nucleic acid extraction from human biological samples. Methods Mol Biol. 2019;1897: 359–383.
4. Forensic SP. Forensic spermatozoa detection. Am J Forensic Med Pathol. 2019;40:304–311.
5. Aparna R, Iyer S, Kumar N, et al. Forensic DNA profiling of tears stains from commonly encountered substrates. Forensic Sci Int. 2021;328:111006.
6. Bholeai B, De Jong BJ, De Puit M, et al. Effect of common fingerprint detection techniques on subsequent STR profiling. Forensic Sci Int Supplement Series. 2011;3:e429–430.
7. Gino S, Omedei M. Effects of the most common methods for the enhancement of latent fingerprints on DNA extraction from forensic samples. Forensic Sci Int Genet Suppl Ser. 2011;3: e273–e274.
8. Thamnurak C, Bunakkharasawat W, Rienfrojpitak S, et al. DNA typing from fluorescent powder dusted latent fingerprints. Forensic Sci Int Supplement Series. 2011;3:e524–525.
9. Pérez-Vergara D. Las células epiteliales: evidencia importante en casos forenses. Gac Int Cienc Forense. 2017;24:20–33. Spanish.
10. Kanokwongnuwut K, Kirkbride KP, Kobus H, et al. Enhancement of fingermarks and visualizing DNA. Forensic Sci Int. 2019;300: 99–105.
11. Hefetz I, Einol N, Faerman M, et al. Touch DNA: the effect of the deposition presurre on the quality of latent fingermrks and STR profiles. Forensic Sci Int. 2019;38:105–112.
12. Kaesler T, Kirkbride KP, Linacre A. DNA deposited in whole thumbprints: a reproducibility study. Forensic Sci Int Genet. 2022;58:102683.
13. Ostojic L, Wurmbach E. Analysis of fingerprint samples, testing various conditions for forensic DNA identification. Sci Justice. 2017;57:35–40.
14. Fischer H, Scherz J, Szabo S, et al. DNase 2 is the main DNA-degrading enzyme of the stratum corneum. PloS One. 2011;6:e17581.
15. Eckhart L, Lippens S, Tschachler E, et al. Cell death by cornification. Biochim Biophys Acta. 2013;1833:3471–3480.
16. Costanzo A, Fausti F, Spallone G, et al. Programmed cell death in the skin. Int J Dev Biol. 2015;59:73–78.
17. Syndercombe CD. Mitochondrial DNA in forensic use. Emerg Top Life Sci. 2021;5:415–426.
18. Lee HY, Kim NY, Park MJ, et al. A modified mini-primer set for analyzing mitochondrial DNA control region sequences from highly degraded forensic samples. Biotechniques. 2008a;44: 555–558.
19. Schrader C, Schielke A, Ellerbroek L, et al. PCR inhibitors — occurrence, properties and removal. J Appl Microbiol. 2012;113: 1014–1026.
20. Thomasma SM, Foran DR. The influence of swabbing solutions on DNA recovery from touch samples. J Forensic Sci. 2013;58:465–9.
21. Burrill J, Daniel B, Frascione N. A review of trace "touch DNA" deposits: variability factors and an exploration of cellular composition. Forensic Sci Int Genet. 2019;39:8–18.
22. Romano CG, Mangiaracina R, Donato L, et al. Aged fingerprints for DNA profile: first report of successful typing. Forensic Sci Int. 2019;302:109905.
23. Pfeifer CM, Klein-Unseld R, Klintschar M, et al. Comparison of different interpretation strategies for low template DNA mixtures. Forensic Sci Int Genet. 2012;6:716–722.
24. Manoli P, Antoniou A, Bashiardes E, et al. Sex-specific age association with primary DNA transfer. Int J Leg Med. 2016;130: 103–112.
25. Goray M, Fowler S, Szkuta B, et al. Shedder status. An analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time. Forensic Sci Int Genet. 2016;23: 190–196.
26. Fonneløp AE, Ramse M, Egeland T, et al. The implications of shedder status and background DNA on direct and secondary transfer in an attack scenario. Forensic Sci Int Genet. 2017;29: 48–60.
27. Lowe A, Murray C, Whitaker J, et al. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. Forensic Sci Int. 2002;129:25–34.
28. Phipps M, Petricevic S. The tendency of individuals to transfer DNA to handled items. Forensic Sci Int. 2007;168:162–168.
29. Aase JM, Lyons RB. Technique for recording dermatoglyphics. Lancet London (UK). 1971;1:432–443.
30. Kogelnik AM, Lott MT, Brown MD, et al. MITOMAP: a human mitochondrial genome database. Nuc Acids Res. 1996;24: 177–179.
31. Brandon MC, Ruiz-Pesini E, Mishmar D, et al. MITOMASTER: a bioinformatics tool for the analysis of mitochondrial DNA sequences. Hum Mutat. 2009;30:1–6.

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