

STR genotyping and mtDNA sequencing of latent fingerprint on paper

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Abstract

A systematic study was conducted to investigate whether DNA can be successfully extracted from latent fingerprints deposited on ordinary paper and analysed using short tandem repeat profiling and mitochondrial DNA sequencing. In order to evaluate the performance of latent fingerprint analysis in a criminal case, experiments with varying conditions were carried out to improve our understanding of low copy number (LCN) DNA typing. After optimising the extraction methods to achieve increased sensitivity, the examination of touched paper can routinely yield the STR profile of the individual who has touched it. A fingerprint can therefore be considered as a potential source of DNA for genetic identification. Nevertheless, the findings of our “after enhancement experiment” (using chemically or physically pre-treated fingerprints), and our “mixture experiment” (using fingerprints from three to four people on the same sheet of paper) help to define the limitations of the low copy number PCR technique in forensic casework.

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1. Introduction

The polymerase chain reaction enables the amplification of template material from minimal amounts of extracted DNA. The development of multiplex STR primer systems, allowing the simultaneous amplification and separation of more than 10 STR loci in a single analytical procedure, and automated DNA fragment analysis provide the possibility to obtain a DNA profile from almost any source of biological material. Thus, now only one nucleated buccal cell [1,2], one telogen hair [3,4], saliva stains on cigarette butts [5,6], postage stamps and envelope sealing flaps [6–10], epithelial cells from excessively pressured fingerprints during strangulation [11,12] or even latent fingerprints applied to drinking glasses [13], clothes [14] and various other objects [15], may be adequate for DNA profiling. In addition to studies

using latent fingerprints we report on the detection of DNA profiles from fingerprints deposited on ordinary sheets of paper which may be relevant in many crime scenarios, e.g. involving fraud or blackmail.

The main goal of our investigation was to increase the sensitivity and effectiveness of the extraction and amplification methods and at the same time provide a systematic series of experiments considering different conditions influencing the deposition and the detection of fingerprints on paper.

2. Materials and methods

2.1. Sample preparation

Commercial office printer paper was used as starting material for all experiments. We selected a commonly used paper type, a recycled paper with 80 g/m² mass and 77–78%

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Table 1
Six experiments on latent fingerprints deposited onto paper by touching

Experiment	Condition	Number of samples
Touching period (s)	60	4
	50	11
	30	4
	10	4
	5	14
	2	4
	1	8
Delay (day)	5	5
	3	4
Time of day	Morning	1
	Noon	2
	After sport	2
	Evening	2
Swab	Cotton swab	4
	Swabbed paper	2
Enhancement	Ninhydrin	5
	Iodine	4
	Soat	2
Mix stain	3 mixture	4
	4 mixture	4

white ISO. First of all, 5 cm × 2 cm sections of paper were prepared for all experiments. Four donors, two male and two female touched the prepared paper sections between the thumb and the index finger in the same manner and with the same pressure as when turning over pages of a book or magazine. The fingerprints were collected from each person separately for each sample with a waiting period between samples of at least 1 day.

In order to evaluate the performance of latent fingerprint DNA typing for forensic genetics six separate experiments were carried out (Table 1).

1. In the *touching period experiment* paper was touched by the four donors for seven different handling time periods ranging from 1 to 60 s. The donors were allowed to wash their hands in the morning as usual, but not immediately before collecting the samples. This experiment should demonstrate whether the quantity and quality of DNA profiles recovered from latent fingerprints are dependent upon the respective touching period and upon the subject who provided the fingerprint. Several groups of researchers have shown that the amount of DNA appears to be donor-dependent [12,15–18].
2. In the *delay experiment* the samples were prepared by touching the paper for 5 s and stored for 3 or 5 days at room temperature before DNA extraction.
3. The *experiment time of day* was introduced to determine whether there is a difference between fingerprints

prepared in the morning, during the course of a day at noon, after sports and in the evening. For this experiment all papers were touched for 5 s.

4. The *swab experiment* was carried out to investigate the possibility of removing latent fingerprints from paper using a cotton wool swab without destroying the paper. All other extractions were carried out by cutting the paper into small fragments. The latent fingerprints for the swab experiment were deposited by touching the paper for 2 and 10 s, respectively.
5. The *experiment after enhancement* aimed to determine the effect of two chemical (ninhydrin and iodine) and one physical (soat) fingerprint visualisation enhancement procedures on DNA analysis of latent fingerprints deposited on paper surfaces for 2 and 30 s, respectively.
6. The *mixture experiment* was designed to investigate a scenario with overlapping fingerprints from several individuals. Eight samples were prepared, four samples with fingerprints from three donors and four samples with four donors. Contrary to studies dealing with two-person mixtures [19,20], the aim of this experiment was not to identify the donors of the mixed biological stain but to test whether the donor who touched the paper last would provide quantitatively and qualitatively the best DNA profile.

In addition, several selected extracts from the *touching period experiment* were subjected to *mtDNA* HV-1 amplification and DNA sequencing to investigate whether samples which did not yield a STR profile could provide *mtDNA* sequence results.

3. Extraction method

After the best applicable extraction technique was determined in an initial study [21], all extractions were carried out using the commercial InViSorb™ Forensic Kit (Invitex). The extraction kit is based on a silica particle suspension matrix (InViSorb 50™ carrier), which binds cellular DNA. Some steps of the producer's protocol were modified to achieve the best performance in terms of sensitivity.

To facilitate the extraction each handled piece of paper was cut into small fragments and transferred into a 2 ml Eppendorf tube (the four samples for the *swab experiments* were not cut but extracted from cotton swabs). One milliliter of Lysis Buffer D (50 °C) was added to the sample. The solution was incubated at room temperature for 4 h in a rotor, followed by centrifugation for 4 min at 20 000 × *g*. The supernatant was transferred into a new 1.5 ml Eppendorf tube. Fifteen microliters of carrier suspension were added to the supernatant, followed by incubation for 20 min at room temperature in a rotor with rotation to enhance binding of the DNA to the silica particles. The solution was vortexed thoroughly twice during incubation. Following incubation the sample was centrifuged for 3 s at 8000 × *g*

so that the carrier would form a pellet, after which the supernatant was discarded. The DNA-binding carrier suspension was rinsed twice with 1 ml wash buffer containing 96% EtOH and centrifuged at $20\,000 \times g$. Subsequently, the carrier suspension pellet was dried, and then resuspended thoroughly in 50 μ l of Elution Buffer D (70 °C) by vortexing. The tube was incubated at 60 °C in a water bath for 20 min to elute the DNA. During incubation the solution was vortexed thoroughly twice until the pellet was completely resuspended. After incubation, the solution was centrifuged for 4 min at $20\,000 \times g$ and then the DNA containing supernatant was transferred into a fresh 1.5 ml tube. The DNA extracts were always centrifuged for 3 min at $20\,000 \times g$ prior use.

4. Amplification

All amplifications were carried out in an Eppendorf Mastercycler.

4.1. Nuclear DNA

The DNA extracts were amplified using the commercial AmpF/STR[®] Profiler Plus[™] PCR Amplification Kit (Applied Biosystems).

The amplification was conducted in a 25 μ l final reaction volume containing 10 μ l AmpF/STR[®] PCR Reaction Mix[™], 5 μ l AmpF/STR[®] Profiler Plus[™] Primer Set, 2.5 U of AmpliTaq Gold[™] and 3 μ l DNA extract. After the PCR optimisation, where the initial denaturation time (5, 7 and 11 min) and the number of cycles (28, 32 and 38) were varied, all STR amplifications were carried out under the following conditions: initial denaturation at 95 °C for 7 min, followed by 38 cycles at 94 °C for 1 min, 59 °C for 1 min, 72 °C for 1 min and 60 °C for 45 min final extension.

4.2. Mitochondrial DNA

mtDNA was amplified using a primer pair designed specifically for degraded DNA [22] using the Primer Select[™] software (Lasergene, DNA Star). Table 2 displays the primer pair with information on primer sequences, primer annealing temperatures and product length.

The amplification was conducted in a 50 μ l final reaction volume, containing 1 \times PCR Gold Buffer (Applied Biosystems), 1.5 mM MgCl₂, 2.5 U AmpliTaq Gold[™] (Applied Biosystems), 0.2 μ M forward primer, 0.2 μ M reverse primer

and 3 μ l DNA extract. The amplification conditions were: 94 °C for 4 min followed by 32 cycles of 94 °C for 45 s, 55 °C for 30 s, 72 °C for 3 min and the final extension at 72 °C for 10 min. Prior to sequencing the PCR product was purified using the commercially available QIAquick[™] PCR Purification Kit (Qiagen). Sequencing of the purified products was carried out using the fluorescent dye labelling cycle sequencing technique according to the supplier's protocols on an ABI 310 automated DNA sequencer. Cycle sequencing was performed using 4 μ l Big Dye[™] Ready Mix (Applied Biosystems), 3 pmol forward or reverse primer, 2–4 μ l purified PCR product and HPLC water qs to 20 μ l for each sample. The Eppendorf Mastercycler was used with the following conditions: 25 cycles at 96 °C for 20 s, 55 °C for 15 s and 60 °C for 4 min.

5. Electrophoresis

The amplification products were first detected and quantified by electrophoresis using a 2% agarose gel and ethidium bromide staining. In order to analyse the amplified Profiler Plus[™] and cycle sequencing products a fluorescent-based capillary electrophoresis was carried out using the ABI Prism 310 Genetic Analyzer and evaluated using GeneScan[®] 3.1 and Sequencing Analysis[™] 3.0 software (Applied Biosystems).

6. Interpretation of STR Profiles

An allele was scored when its peak height was >5% of the peak height of the most prominent allele at a given locus. False alleles were called when they were >5% of the most prominent allele, but were not present in the genotype of the donor, as determined from a buccal swab control DNA sample. Stutter bands were also scored as false based on this guideline.

The proportion of successfully typed profiles excludes all loci affected by allele or locus dropout, but not the occurrence of false alleles, which have been scored separately. Thus, a complete profile was scored when the correct alleles were detected for all 10 loci (Tables 3 and 4).

Successfully typed alleles were calculated as the proportion of the total number of expected alleles at a given locus for all individual assays of a series of experiments; e.g. 49 separate amplifications were performed for seven *touching period experiments* so that a maximum of 98 individual

Table 2

The HV-1 mtDNA primer pair with information on primer sequences, positions, primer annealing temperatures and product length

Primer	Position	Annealing temperature (°C)	Product length (bp)
5'-TACATTACTGCCAGCCACCAT-3'	16097–16177	55	271
5'-ATGGGGACGACGAGAAGGGATTG-3'	16348–16368	55	271

Table 3

DNA profiles (arranged according to increasing fragment length) recovered for the entire *touching period examination* including four donors and seven different handling periods

	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18	Mean
Profile	0.97	0.91	0.92	0.92	0.93	0.91	0.90	0.87	0.73	0.71	0.88
Allele dropout ^a	0.03	0.09	0.08	0.08	0.07	0.09	0.1	0.13	0.26	0.28	0.12
Locus dropout	0.01	0.02	0.02	0.02	0.02	0.03	0.04	0.04	0.1	0.1	0.04
False alleles	0.01	0.09	0.08	0.06	0.04	0.07	0.04	0.01	0.00	0.02	0.04

^a Includes missing alleles from locus dropout.

Table 4

Proportions of successfully obtained DNA profiles from all different experiments; the proportion of the loci regarding allelic dropouts and the false alleles are also listed

	Experiment				
	Touching	Delay	Time of day	Swab	Enhancement
Profile	0.88	0.86	0.93	0.80	0.47
Allele dropout ^a	0.12	0.14	0.07	0.20	0.53
Locus dropout	0.08	0.06	0.01	0.07	0.21
False alleles	0.05	0.03	0.01	0.02	0.16

^a Includes missing alleles from locus dropout.

alleles are to be expected for each locus. When the results for all loci of the multiplex were compared between experiments or persons, the median of the respective proportions was calculated (Figs. 1 and 2).

7. Results

Eighty-eight percent of the DNA profiles were recovered for the complete *touching period examination* including four donors and seven different touching periods (Table 3,

last column). The proportion of DNA profiles recovered for the 10 loci of the Profiler Plus™ Kit, arranged according to increasing fragment lengths, the number of locus and allele dropouts and the proportion of false or extra alleles are shown in Table 3. A slight decrease in the successful detection of longer fragments can be observed. The STR profile depicted in Fig. 3 exhibits strong signals for the smaller fragments and a clear decrease in peak height for longer fragments. This pattern was typical for all the STR profiles obtained.

The proportion of DNA profiles deposited on paper for the seven time intervals is given in Fig. 1. The latent fingerprints

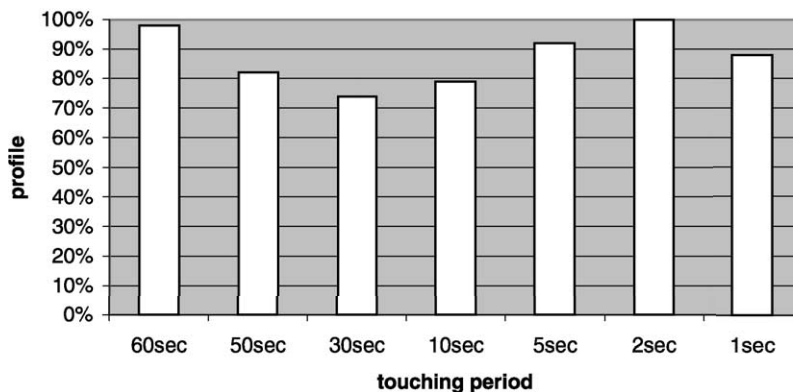


Fig. 1. Proportion of DNA profiles obtained from the entire *touching period examination* deposited on paper for the seven time intervals (1–60 s).

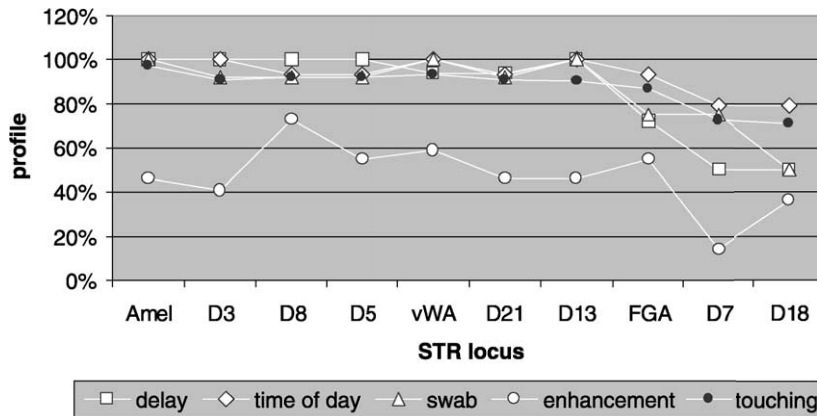


Fig. 2. The proportion of successfully typed DNA profiles from all different experiments for the 10 loci of the Profiler Plus™ Kit, sorted according to the increasing fragment lengths.

prepared by touching the paper for 60 s as well as for 2 s have yielded a complete STR profile indicating that the proportion of successfully detected DNA profiles is not dependent on touching period (Fig. 1).

Fig. 2 summarises the proportion of successfully typed DNA profiles from all experiments, sorted according to increasing fragment length of the STR loci. Figure underlines that the pre-treated fingerprints provided only partial profiles, in comparison with those from other experiments.

The proportions of successfully typed DNA profiles for all experiments are shown in Table 4 together with the proportion of allele and locus dropouts and the occurrence of false alleles. High rates (80–93%) of correctly typed profiles were obtained from the swab (80%), delay (86%), touching (88%) and time of day experiments (93%). In contrast, only 47% of the profiles from fingerprints treated with enhancement chemicals could be typed. The mixture experiments composed from three or four subsequent donors

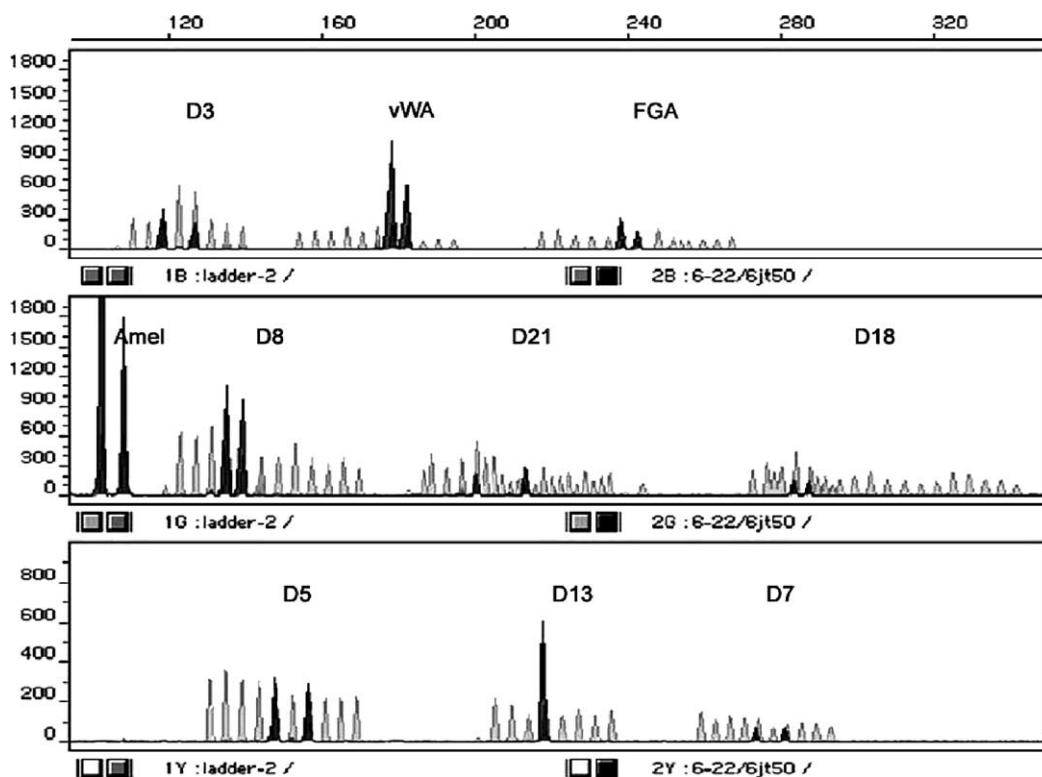


Fig. 3. Typical STR profile from 50 s latent fingerprint, carried out with 38 PCR amplification cycles.

Table 5
mtDNA sequencing results of the selected samples (from individuals A–D) in comparison with the obtained STR profiles of the same extracts

Samples (s)	STR profiles (%)	mtDNA
A 50	80	+
D 5	95	+
D 10	65	+
D 50	100	+
C 30	100	+
C 5	55	+
B 2 ^a	0	+
B 5	70	+
B 5	100	+
B 10	55	+

^a This sample is a 2 s latent fingerprint treated with ninhydrin and derives from the after enhancement experiment.

did not show any tendency to indicate the donor depositing the final fingerprint (data not shown).

Table 5 summarizes the results of mtDNA amplification of the 271 bp HV-1 fragment and sequencing of the selected samples in comparison to the STR profiles obtained by analysing the same extracts.

All samples could be successfully sequenced, even those which yielded no or only a partial STR profile (0–70%).

8. Discussion

Our findings show that it is possible to obtain DNA profiles from latent fingerprints left by simple skin contact on objects and likewise on ordinary sheets of paper. These results clearly extend the observations described by other authors on low copy number (LCN) PCR results from latent fingerprints recovered from glass, textiles, skin and other surfaces [11,13–15,18,23]. The DNA extraction procedure has been optimised in order to achieve the best performance in terms of sensitivity. Thus, the examination of touched paper can routinely yield the STR profile and mtDNA sequence of the individual who has touched it independent of the handling time. The observation made by others that individuals differ in their tendency to deposit DNA [18] does not appear to be relevant to the same extent in our investigation, either due to the sensitivity of our extraction procedure or to the fact that there were no significant differences among the four donors of the study. The results of the *touching period examination* show that the latent fingerprints from the four donors yielded approximately the same percentage of STR profiles (data not shown), although an individual difference in terminal epidermal cell differentiation is thoroughly expected. The differentiation process regulating the epidermal growth is accompanied by changes in cellular biochemistry involving the activation of catabolic enzymes. The enzyme which is responsible for DNA degradation is a

putative Ca²⁺/Mg²⁺-dependent endonuclease that fragments the DNA into approximately 200 base pair multimers [24,25]. The size-dependent decrease of successfully typed profiles observed in the *touching period experiment* may confirm the mechanism described by these authors. The findings depicted in Table 3 show that loci D7 and D18 with fragment lengths between 250 and 341 bp exhibited a 20% reduction compared to the other loci with shorter fragment lengths.

Further examinations show that latent fingerprints prepared 3 or 5 days before extraction, in the morning, at noon, after sport, or in the evening also provided in most cases a full or nearly full DNA profile (86–100%). The *time of day experiment* shows no significant differences between fingerprints deposited in the morning without prior hand washing and during the normal course of a day.

The results of *swab experiment* demonstrate that the epithelial cells can be swabbed from paper with a sterile moistened cotton swab and that the DNA can be extracted when documents are submitted as exhibits in a casework. During the *swab experiment* we observed that the biological material of a fingerprint cannot be completely removed from paper. Two samples which were typed again from two previously swabbed paper sections still yielded 100% profiles. That means, if it is legally admissible it would be possible to use the “cut to pieces” method when the swab method fails to deliver a result.

The high sensitivity and effectiveness of the method may not only be a result of the improved extraction technique but can also be attributed to the LCN PCR technique applied. The sensitivity of the PCR has been increased by subsequently raising the number of cycles (28, 32 and 38). The successful use of the LCN method with an increased number of PCR amplification cycles has also been described by other authors. For example, van Hoofstat et al. [26] analysed fingerprints with 28–40 cycles, Barbaro et al. [4] analysed hair shafts using 35–43 cycles. In palaeoanthropology laboratories, increased PCR cycle numbers up to 50 are routinely used to amplify ancient DNA from bones [27,28]. The benefit of increased sensitivity derived from raising the number of amplification cycles has to be balanced against a reduction of profile quality. Our investigation shows that PCR with 38 cycles proved to be the most effective in contrast to 32 and 28 cycles (data not shown). Increasing the number of amplification cycles to 38 did not raise the incidence of artefacts and stutters, thus the quality of results was not compromised (Fig. 3).

For mtDNA analysis, we were able to obtain complete results from PCR fragments amplified using only 32 cycles. This lower number of cycles correlates well to the higher copy number of mtDNA molecules which is the main reason for the 100% success rate of HV-1 sequence analysis compared to nuclear DNA STR typing.

Nevertheless, the highly sensitive system that may reveal DNA from any source leads to a higher contamination risk and cannot be applied without the strongest contamination

prevention guidelines. We considered all potential sources of contamination and avoided them, in addition to the guidelines given by Gill [29], by using the following precautions:

1. Pre-PCR and post-PCR work was carried out not only in separate laboratories but separate buildings.
2. The flow of experiments from one building to another was strictly controlled in one direction; from pre-PCR to post-PCR areas only.
3. The pre-PCR laboratory area could only be entered through two separate security locks.
4. In the first lock street wear was removed and in the second lock the special security laboratory clothes were put on. This included two pairs of gloves, face mask, complete protective clothing, hair hood, overshoes and protective visor.
5. Sensitive working areas and equipment were UV irradiated for 4 h at night. Additionally, all reaction tubes and pipet tips were irradiated with UV for at least 1 h prior to use.
6. All rooms were regularly washed with bleach. Every item entering these rooms was washed with bleach and subsequently UV irradiated. Filtered water for cleaning and HPLC water for PCR were UV treated for at least 10 h.

Despite the high cycle number used there was no evidence of contamination. Negative PCR controls without DNA were included for all experiments and never produced any amplified fragment detectable by agarose gel or fluorescent capillary electrophoresis.

Typically, in forensic casework, the latent fingerprint is invisible and therefore difficult to locate, which could make it unlikely to isolate only the offender's fingerprints. The localisation problem could be solved by treating the fingerprint with chemical enhancers for visualization prior to DNA extraction. However, in contrast to other authors, it was observed in the *after enhancement experiment* that following treatment with chemical or physical methods fingerprints could only be typed successfully for an average of 47% of the profiles. A number of authors [30–32] have demonstrated that previous treatment of items have no negative effect on DNA profiling by analysing, e.g. bloody fingerprints or saliva for their investigations. Therefore, they only provide evidence for the fact that typing is possible after treatment with classical latent fingerprint techniques in cases when sufficient amounts of genetic material is available. The higher level of false alleles (16%) observed in the *enhancement experiment* compared to the other four experiments (Table 4) could have resulted from different procedures for those samples. Whereas the first four series of experiments were carried out entirely in the DNA-free environment of our pre-PCR laboratory (see above), the enhancement procedures were performed in a routine police laboratory using standard equipment and solutions.

The aim of our *mixture experiment* was the detection of the *last person* who touched the paper, not the discrimination

of the genotypes of all contributors. However, we could not draw conclusions about the number of donors involved from any of the mixed stains generated here, on the basis of the allele quantity. The results of the *mixture experiment* supports the observations of van Oorschot and Jones [15] in that the strongest profile obtained was not always that of the person who last touched the object. Gill et al. [33] have developed an approach based on likelihood ratios for the interpretation of mixtures, however, this method assumes that the alleles of all contributors are present and can be detected.

In the context of low copy number PCR conditions, a set of guidelines has been proposed [34] to correctly identify the profile of a stain donor. The most important guideline for reporting results from LCN experiments is to confirm the presence of an allele by replication. We carried out replication experiments from selected DNA extracts and found that the profiles could be reproduced reliably (data not shown).

In summary, systematic studies with known reference profiles have generated reliable and reproducible results from fingerprints deposited on paper. In practical casework, however, unknown and varying circumstances will make the evaluation of a genetic fingerprint of an unknown person more difficult. These can especially be misleading due to additional alleles introduced via secondary or tertiary transfers [18] as well as information deficits caused by allele dropouts.

We think that the protocols presented here can be used in practical casework, though many scenarios might be more complex than the controlled laboratory situation. However, a latent fingerprint is always a LCN DNA case and the observation of the LCN criteria including the contamination prevention measures as outlined above are necessary for successful and reliable analysis.

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