

## Efficiency of forensic mtDNA analysis Case examples demonstrating the identification of traces

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### Abstract

The paper presents results of forensic mitochondrial DNA analyses which were aimed at typing the traces caused by touching or abrasion of skin cells. Five cases of strangulation tool investigation are summarised. Two cases of homicide could be cleared up by identifying the mtDNA of both the victim and the suspect on cables which had obviously been used as strangulation tools. In eight of 10 cases, weapons could be reliably assigned to their users. The mtDNA of the users could be even detected on cartridges after firing. In one case, evidence of a suicide could be provided by means of mtDNA sequencing of the wiping traces on a suicide note. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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### 1. Introduction

Since the development of classical fingerprint analysis at the end of the 19th century (for a summary see Ref. [1]) this method has often been employed successfully for identifying users of objects. However, a number of limitations have turned out when using this method for the identification of traces. DNA techniques have offered new chances for identification in that they permit the analysis even of other traces left on objects. PCR typing of mucoid cells from buccal cells and saliva has become a well established method and is often employed for analysing biological stains, e.g., on cigarette butts and bite marks [2–4]. Other epithelial cells which have become important are those that are transferred

through scratching, i.e., debris from finger nails [5] and human dandruff [6,7]. In other investigations it was demonstrated that epidermal cells were exchanged by physical contact of persons from skin to skin [8,9]. Van Oorschot and Jones [10] reported the possibility to recover “DNA fingerprints from fingerprints”.

In comparison with nuclear DNA, the number of copies of mitochondrial DNA (mtDNA) in cells is much higher. Hence, mtDNA can often be typed more successfully than nuclear DNA, in particular in stains with a low DNA load. Sequencing techniques for analysing the mitochondrial control region for forensic purposes and population data have been presented in the literature [11–17].

This paper reports five cases in which traces on strangulation tools were investigated. We were requested to examine pistols in three criminal cases and find out whether they exhibited traces of the users in question. We took this opportunity to conduct an extensive study on the identification of

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users of weapons and summarised the analyses of traces recovered from 10 police pistols and cartridges. Finally, this paper also reports a case in which we succeeded in verifying the writer of a suicide note by employing molecular methods.

## 2. Materials and methods

### 2.1. Investigation of traces on strangulation tools

We examined strangulation tools in five cases with the aim to establish whether the strangulation tools exhibited both traces of the victim and traces of the perpetrator.

#### Case no. 1

A telephone cable was found on the floor near a strangulation victim. As reference material we had blood of the victim and saliva samples of three suspects available.

We cut the 2-m long cable into four sections and wiped them separately using a wet glass fleece. Sections 2 and 3 were taken from the middle, and Sections 1 and 4 were the two ends of the cable.

#### Case no. 2

The situation was similar to that in the first case, but involved an electric cable found in the apartment of the suspect. Hence, investigation was aimed at detecting traces of the victim on the cable. Again, we cut the cable into four sections and looked for traces of the victim and of the perpetrator.

#### Case no. 3

A girl was hanged by means of a hemp rope after having been killed. We cut the rope into a number of sections. Preferably those regions where the perpetrator had tied knots were rinsed with about 5 ml water each. The rinsing water was vigorously centrifuged. Then, the sediment was analysed using the proteinase K/phenol extraction method as described below.

#### Case no. 4

A nylon cord of a curtain was used as the strangulation tool in a case of murder and robbery. To recover and analyse the traces, the cord was cut

into sections of 15 cm length each which were rinsed with distilled water. The rinsing water was centrifuged at 10 000×g. DNA was extracted from the sediment by proteinase K/phenol extraction as described below.

#### Case no. 5

A woman claimed that she had been raped and strangled before she was able to escape. The strangulation tool, i.e., an electric cable, was presented. However, the statement of the victim involved several contradictions and the suspicion arose that the woman only pretended to have been assaulted. We tried to recover skin traces as described below.

### 2.2. Investigation of traces on firearms

After target practice 10 police officers gave us their pistols and five cartridges each as well as samples of their saliva. The cartridges belonged to the ammunition which the officers had personally loaded into the magazines of their pistols.

As shown in Fig. 1 we analysed DNA from five areas of the pistols where fingers and/or palms usually leave the strongest impression. These areas are the trigger cock, the grip, the hammer, the magazine, and the cartridges.

### 2.3. Investigation of traces on folded paper

In one case we identified the author of a note by means of the traces he left on it. The circumstances suggested that a person who was found dead had committed suicide. A small note containing a few lines was found. Speculations questioning the suicide came up and we were requested to issue a DNA expertise complementing the graphological expertise of the suicide note. It was our task to recover traces of the dead person in order to clarify whether the note had possibly been folded by another person. We wiped the creases of the folded note with glass fleece and sequenced the mitochondrial DNA.

Thereafter, we repeated the test of recovering finger traces on creases of paper with two members of our staff. One of these tests is demonstrated below.



Fig. 1. Five areas of a pistol which were wiped with glass fleece to recover DNA traces: (1) trigger cock; (2) grip; (3) hammer; (4) magazine; (5) cartridge.

#### 2.4. DNA extraction

For recovering DNA traces we followed the procedure presented by Wiegand and Kleiber [8]. Skin cells and epithelial cell debris were recovered from the objects of interest (as described in the cases above) using pieces of glass fleece (application tabs, Amersham-Pharmacia, Freiburg, Germany) moistened with distilled water.

DNA was extracted from stain samples as well as saliva or blood samples from the suspects and the victim by almost identical methods utilising a modified version of a protocol presented by Old [18]. Four hundred  $\mu\text{l}$  of lysis buffer containing 150 mM NaCl, 25 mM  $\text{Na}_2\text{EDTA}$ , 10 mM Tris-HCl (pH 8.5), 1% SDS and 20  $\mu\text{l}$  proteinase K (20 mg/ml) were added to the samples and incubated for 16 h at 37°C. Subsequently, the samples were extracted with phenol and chloroform. Ammonium acetate was added to a final concentration of 500 mM. DNA precipitation was performed with absolute alcohol (all of these agents were from Merck, Darmstadt, Germany). For trapping the minimal DNA quantities expected in stains, 1  $\mu\text{g}$  ribosomal *E. coli* RNA (Sigma-Aldrich, Deisenhofen, Germany) was added prior to phenol/chloroform extraction when DNA was extracted from stain samples. DNA preparation was not followed by a DNA quantification test.

#### 2.5. Autosomal STRs

Autosomal STRs were analysed by investigating the well-established loci HumFGA, HumTH01 and HumACTBP2 using commercial kits and/or ladders (Serac, Bad Homburg, Germany).

#### 2.6. Amplifying mitochondrial D-loop fragments

For mitochondrial sequencing of the reference material (blood and saliva), a full sequencing strategy was used by sequencing PCR products of the entire D-loop. They were amplified with the primers L15926 (5'-TAC ACC AGT CTT GTA AAC C-3') and H00652 (5'-AGA AAG GCT AGG ACC AAA CC-3'). Since stains from fingers and palms are often partially degraded, short PCR fragments were generated for sequencing using closer spaced primer pairs, e.g., L15990 (5'-TTA ACT CCACCA TTA GCA CC-3') and H16498 (5'-CCT GAA GTA GGA ACC AGA TG-3') for amplifying hypervariable region 1 (HV I) fragments and L00029 (5'-GGT CTA TCA CCC TAT TAA CCA C-3') and H00408 (CTG TTA AAA GTG CAT ACC GCC A) for producing HV II fragments, respectively. The 3'D-loop  $(\text{CA})_n$  repeat polymorphism [19,20] located in HV III [21] was amplified using the primers L00484 (5'- CTC CCA TAC TAC TAA TCT CA-

3') and H00537 (5'-TGG TTG GTT CGG GGT ATG-3'). Primers were synthesised by Eurogentec (Seraing, Belgium). Amplification was performed (after a first denaturation step of 95°C, 3 min) for 30 cycles of 60 s at 94°C, 60 s at 56°C, and 60 s at 72°C (final extension, 10 min, 72°C) using a PTC 200 Multicycler (MJ Research, Watertown, USA). The total volume was 25 µl containing 0.21 ng DNA, 200 µM each dNTP (Sigma–Aldrich), 0.4 µM each primer, 1×PCR buffer, 1.5 mM MgCl<sub>2</sub>, and 1 unit Goldstar DNA Polymerase (Eurogentec).

### 2.7. Sequencing of mitochondrial DNA

To remove excessive primers and nucleotides from the PCR reaction prior to sequencing we used the Qiaquick PCR purification kit (Quiagen, Hilden, Germany). All sequencing tools, e.g., 373A sequencer and the RR Terminator Cycle Sequencing Ready Reaction Kit, were used from Perkin-Elmer-ABI (Foster, USA).

Each sample was sequenced twice using L15990 and L00029 as sequencing primers. Reaction was carried out in a final volume of 12 µl containing 2.5 µl reaction mix, 1.5 µl primer (1 pmol/µl) and 40–80 ng PCR product. Cycling conditions (25 cycles) were: 5 s at 95°C, 90 s at 60°C and 90 s at 50°C.

## 3. Results

### 3.1. Identification of victim and perpetrator by investigating traces on strangulation tools

As a result of a close physical contact between exposed skin and objects, epidermal cells may be transferred onto the objects. As could be demonstrated, such traces from the neck of the victim and the exposed hands of the perpetrator can be recovered and typed by carefully wiping the strangulation tools, such as cables, with moistened glass fleece. In case no. 1 (described above) we succeeded in identifying both the strangulation victim and the perpetrator by mitochondrial sequencing. Traces which were recovered from the middle part of the telephone cable (Section 3) matched the victims' profile, whereas the DNA pattern obtained from

traces on one end of the cable (Section 1) matched the mitochondrial sequence of the suspect (Table 1). When we investigated the STRs TH01, FGA and ACTPB2 in this case, we obtained sufficient results from Section 3 only; however, not from the ends of the cable (not shown).

Table 2 contains a summary of the investigation results of five forensic strangulation cases (including case no. 1 described above). As assumed before, mitochondrial sequences can be obtained more often than STR patterns. Furthermore, the chances of successfully typing the skin cells from the neck of the victim seem to be better than those of identifying the perpetrator by analysing hand and finger traces. In case no. 2 we could only identify stains from the victim on the antenna cable and did not detect any traces from the perpetrator. As the police had found this cable in the flat of the suspect, this result was of high evidentiary value.

The outcome of the investigation of the hemp rope in the third strangulation case was completely insufficient. The DNA results could not contribute to solving the case because we detected the pattern of the victim only; however, not the pattern of the perpetrator.

Very good results were obtained in the case where a nylon cord of a curtain was used as the strangulation tool. Due to the rough surface of the material, the yield of skin cells from both the victim and the perpetrator was rich and could be typed without limitations in the STR and mitochondrial control region.

In case no. 5 we could not detect and type stains on the alleged strangulation tool. This outcome confirmed the doubts which had come up as a result of contradictions in the statement of the victim and supported the suspicion that this offence had been pretended.

### 3.2. Identification of firearm users

Table 3 shows the results of identifying users of firearms by stain investigation. Eight of 10 police officers, who provided us with their pistols, could be correctly identified when DNA traces recovered from the trigger cock (no. 1), butt (no. 2), hammer (no. 3), magazine (no. 4), and five cartridges (no. 5) were

Table 1

Stain DNA was yielded from two different regions of a phone cable which had been used as a strangulation tool<sup>a</sup>

Anderson	Victim	Suspect 1	Suspect 2	Suspect 3	Cable section 1	Cable section 3
L 16051 A	–	G	–	–	Failed	Failed
L 16093 T	–	C	–	–	Failed	Failed
L 16126 T	–	–	C	–	–	–
L 16129 G	–	C	–	–	–	–
L 16153 G	–	–	A	–	–	–
L 16183 T	–	A	–	–	–	–
L 16189 T	–	C	–	–	–	–
L 16192 C	T*	Failed	–	–	–	T*
L 16223 C	–	Failed	–	–	–	–
L 16256 C	T*	Failed	–	–	–	T*
L 16270 C	T*	Failed	–	–	–	T*
L 16293 G	–	Failed	–	A <sup>++</sup>	A <sup>++</sup>	–
L 16294 C	–	Failed	T	–	–	–
L 16296 C	–	Failed	T	–	–	–
L 16304 T	C*	Failed	–	–	–	C*
L 16311 T	–	Failed	–	C <sup>++</sup>	C <sup>++</sup>	–
L 16390 G	–	Failed	–	–	Failed	–
L 16399 A	G	Failed	–	–	Failed	Failed
L 00073 A	G*	G	G	–	G	G*
L 00152 T	–	C	–	–	–	–
L 00195 T	–	–	–	T <sup>++</sup>	T <sup>++</sup>	–
L 00200 A	–	–	G	–	–	–
L 00207 G	–	–	–	A <sup>++</sup>	A <sup>++</sup>	–
L 00217 T	–	C	–	–	–	–
L 00263 A	G*	G	G	G <sup>++</sup>	G <sup>++</sup>	G*
L 00309. 1	+C*	+C	+C	+C <sup>++</sup>	+C <sup>++</sup>	+C*
L 00315. 1-	+C*	+C	+C	+C <sup>++</sup>	+C <sup>++</sup>	+C*

<sup>a</sup> The DNA pattern from stains of the cable end (Section 1) matched the profile of the suspect 3 (<sup>++</sup>). Stains which were recovered from the middle part of the phone cable (Section 3) matched the pattern of the strangulation victim (\*).

investigated by mitochondrial sequencing. In two cases the identification of the user failed as the traces were mixed and/or the signals were too weak. This high identification rate is possible when results of all

individual investigations are combined. With a reliability rate of 50%, the best results were obtained by analysing the finger traces from the trigger cock. It is remarkable that our analyses provided good

Table 2

DNA traces believed to stem from the exposed neck of the victim and from the hands of the perpetrator were typed and provided very good (<sup>++</sup>), good (+) and nearly good ( $\pm$ ) results<sup>a</sup>

Case	Strangulation tools	Victim profile		Perpetrator profile		Result
		STR	mtDNA	STR	mtDNA	
1	Phone cable	+	++	Failed	++	Highly sufficient
2	Antenna cable	$\pm$	++	Failed	Failed	Highly sufficient
3	Hemp rope	++	++	Failed	Failed	Non-sufficient
4	Nylon cord	++	++	+	++	Highly sufficient
5	Electric cable	Failed	Failed	Failed	Failed	Sufficient (?)

<sup>a</sup> In some cases no result was obtained. As there was the suspicion in case no. 5 that the offence had just been pretended, the failure of stain amplification may be considered a sufficient result.

Table 3

DNA traces recovered from 10 police pistols and five cartridges each were investigated by mitochondrial sequencing<sup>a</sup>

Pistol no.	Trigger (1)	Grip (2)	Hammer (3)	Magazine (4)	Cartridges (5)				
1	+	+	+	±	+	+	+	±	f
2	±	+	±	+	+	±	f	f	f
3	m	m	f	f	f	f	±	±	f
4	m	m	m	f	f	f	f	f	f
5	m	±	+	f	m	m	f	f	f
6	+	m	m	f	±	±	m	f	f
7	+	m	m	±	+	±	f	f	f
8	±	m	m	+	+	+	f	f	f
9	+	+	f	f	m	f	f	f	f
10	+	+	+	±	+	+	f	f	f
Outcome	50% + 20% ± 30% m	40% +; 10% ± 50% m f	30% +; 10% ± 40% m 20% f	20% + 30% ± 50% f	18% ±; 14% ± 8% m 60% f				

<sup>a</sup> The traces had been caused by the unprotected hands of the firearm users during police target practice. The investigation outcome was classified as reliable identification (+), nearly sufficient result (±), mixed stains (m), and completely non-sufficient result (f=failed).

results in 18% and nearly sufficient results in 14% of all cartridges investigated. (Table 3).

### 3.3. Identification of stains on folded paper

It could be demonstrated that persons folding

Table 4

Stain DNA was yielded from the crease regions of a folded paper<sup>a</sup>

Anderson	Suspect	Letter crease
L 16069 C	T	Failed
L 16126 T	C	C
L 16147 C	T	T
L 16362 C	T	T
L 00073 A	G	G
L 00185 G	A	A
L 00263 A	G	G
L 00295 A	G	G
L 00309.1	+C	+C
L 00326 A	G	Failed
(CA) <sub>n</sub> repeat L 00513-L 00523 (CA) <sub>5</sub>	(CA) <sub>6</sub>	(CA) <sub>6</sub>
STRs		
THOI	6–9.3	6–9.3
FGA	22–24	22–24
ACTBP2	18–27.2	18–27.2

<sup>a</sup> The person who had folded the paper could be identified through the matching pattern.

normal writing paper with exposed fingers leave stains on it which can be typed by means of mitochondrial and STR analysis. When we investigated a case of suicide, we sequenced about 250 bp in the HV1 and HV2 regions from stains recovered from the crease of a note and obtained clear readings which were in good agreement with the sequence of the corpse. Thus, the suspicion that another person involved had folded the suicide note, was not supported.

Table 4 depicts one of two successful tests we performed with members of our staff to identify finger traces from the crease region of folded paper

## 4. Discussion

Wiegand and Kleiber [8] investigated a series of experimental strangulations and could isolate up to 2 ng DNA from swabs which were taken from relevant skin areas of the victim.

Firearms, cut-and-thrust weapons, and strangulation tools, after having been used, normally carry a remarkable load of stains. Van Oorschot and Jones [10] demonstrated that swabs from objects of daily use can carry more than 10 ng nuclear DNA of the user which is sufficient for establishing the DNA pattern for forensic individualisation. Apart from

some few nucleated stratum granulosum cells, many denucleated corneocytes are transferred when a physical force is applied [9]. This observation and the fact that in nucleated cells the share of mtDNA in the mtDNA-to-nuclear DNA ratio is much higher are the reasons why we give mitochondrial analysis high priority in our strategy of identifying users of objects. However, a tool for mtDNA quantification has not yet been available. Semi-quantification by electrophoresis [22] cannot satisfy the demands of stain investigation as it involves high material consumption. Hence, we carried out our investigations without any DNA quantification. Nevertheless, we could retrieve DNA patterns from touching traces with good success employing an empirical dosage of the DNA extract for PCR.

It is not surprising that the analysis on perforating FMJ bullets [23] permits the establishment of a highly indicative DNA pattern. It might be of some significance to criminalistics that the users of weapons not only leave their personal identification patterns on the firearms but often also on cartridges, and that these stains can be typed even after firing.

Mitochondrial analysis offers great potentials for individualisation [12–17]. Often mitochondrial analysis is the more convenient and more successful method, whereas STR typing provides a higher discrimination potential. However, higher sensitivity of mtDNA analysis justifies its application, in particular in stains with a low DNA load. Presently, efforts are made to build up mitochondrial databases for forensic purposes which will permit mtDNA opinions to be used in courts [24]. Thus, the identification of users of weapons, strangulation tools, and objects of daily use by means of mtDNA typing could play an ever increasing role in criminalistics in future.

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