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ORIGINAL ARTICLE

Hot flakes in cold cases

Harald Schneider · Thomas Sommerer · Steve Rand · Peter Wiegand

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Abstract In the past, it was almost impossible for forensic scientists to separate DNA from an undefined number of different individuals in mixed stains where, for example, two or more suspects had handled the same weapon. Such samples often contain complex mixtures with the consequence of ambiguous or inconclusive mixed DNA profiles. Using the method described of comprehensive and/or targeted screening of shed cells adhering to tapings of garments or objects enables such stains to be individualized. To evaluate the method, 500 microscopically selected single skin flakes were analyzed using two different commercial STR kits to compare the success rates for each PCR typing system. The method has been validated for use in routine casework and has been shown to be rapid, sensitive, and reproducible. It can be predicted that many cases in the archives with body tapings, which have not yet been examined will benefit from this new or perhaps more appropriate, reanimated, technical development, and of particular importance are serious crimes, the so-called cold cases. The remarkable forensic value of this simple but time-consuming technique is exemplified by 2 out of approximately 100 cases already successfully solved using this approach.

Keywords Skin flakes · Mixed stains · Cold cases · Body tapings

H. Schneider
Hessisches Landeskriminalamt,
Hölderlinstr. 1-5,
65187 Wiesbaden, Germany

T. Sommerer · P. Wiegand (✉)
Institute of Legal Medicine,
University of Ulm,
Albert-Einstein-Allee 11,
89081 Ulm, Germany
e-mail: peter.wiegand@uniklinik-ulm.de

S. Rand
Forensic DNA laboratory,
University Hospital Antwerp,
Wilrijkstraat 10,
B-2650 Edegem, Belgium

Introduction

There can be no doubt that the advent of modern DNA investigations using PCR has made one of, if not the most valuable, contribution to forensic science ever known. The evidential value of DNA for the identification of the donor of human body fluids is so enormous that other strategies for identification tend to be ignored. Even if a "stain" cannot visibly be seen with the naked eye or even under a microscope, deposited DNA can still be detected. But the power of DNA investigations does not only lie with the identification of the classical blood, saliva, or semen stains. There are still many procedures dating back to the pre-DNA period, which can employ even if they are not routinely or ever used in standard forensic laboratories.

The standard procedure for fiber investigations was, and still is, to tape corpses, items of clothing or any other items of evidential value with a special kind of adhesive tape and to microscopically and painstakingly search these tapings step-by-step noting the position of any fibers suspected of having been transferred from one item to the other [1]. Fiber investigations nowadays play a subordinate role to DNA in forensic investigations, although they have not been completely discarded and can sometimes be of vital importance. However, part of the strategy for fiber investigations, namely the methodical searching of tapings, can

also play an important role in DNA investigations. During this fiber taping procedure, all other biological contact traces will also be collected on the tapings, although 20 years ago, this was never even considered when the taping was originally carried out.

"Every contact leaves a trace" is an old forensic maxim attributed to Edmond Locard (Locard's exchange principle), but never actually written in this form, which is still relevant to modern forensic investigations even, or perhaps more appropriately, especially in the age of DNA investigations.

There are many case scenario situations where searching for evidence of DNA transfer can play a decisive role in identifying contact between two persons [2, 3]. Intensive contact between victim and offender is a very common occurrence in crimes of violence, so it is not surprising that DNA in the form of skin particles can be transferred from one to the other and in both directions. However, in many cases, this is only relevant for transfer from the offender to the victim, as in most cases, only the victim is known and available at the time of examination and the offender is still to be identified. By the time the offender has been found, any DNA which had been transferred in this direction will be long gone.

This article describes a practical approach to the searching strategy for identifying DNA trace contact using several (out of many) successful casework examples where the offender could be identified.

Materials and methods

Single flake selection, DNA extraction, and quantification

DNA was extracted from approximately 500 single putative skin flakes, which were selected and prepared by microscopic examination of prepared adhesive tapings (Neschen AG, Bueckeburg, Germany). DNA extraction was carried out using a routine Chelex protocol [4]. The single flakes were transferred directly into 50 µl of chelex suspension. To optimize cell lysis, 5 µl proteinase K (Qiagen 10 mg/µl) and 2 µl of 10% (*v*/*v*) Tween 20 (Fluka) were added and incubated at 56°C overnight (12 h) using an Eppendorf thermo block (Thermomixer comfort, Eppendorf, Germany). Final heating was performed for 8 min at 95°C.

DNA quantification for each sample was performed using the Quantifiler Human Kit™ (Applied Biosystems).

PCR amplification

Short tandem repeat (STR) typing was performed using up to 7 µl of DNA extract depending on the results of DNA quantification. The optimal DNA concentration of 100–150 pg was the target, but failing this, the maximum volume was used. Each extract was tested using the STR multiplex kits

- SEfiler Plus® (D3S1358, FGA, D8S1179, D18S51, D21S11, TH01, VWA, SE33, D2S1338, D16S539, D19S433, Amelogenin) (Applied Biosystems) [5]
- MiniFiler® (D13S317, D7S820, Amelogenin, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA) (Applied Biosystems) [6].

The same DNA extract was analyzed in parallel with both kits. The amplification protocols and the thermal cycling conditions were used according to the manufacturer's instructions. The PCR volume in the experimental study was reduced to 12.5 µl for all tests. Positive and negative controls were co-analyzed in each amplification reaction. Most of the samples were amplified using 33 cycles but for samples with DNA quantification values >100 pg per PCR reaction, 30 PCR cycles were used.

Analysis on ABI Prism 3130 Genetic Analyzer

Electrophoresis was carried out on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Darmstadt, Germany) and analyzed using the GeneMapper® ID Software v3.2. For comparison purposes, samples were prepared with 10 µl Hi-Di™ Formamide (Applied Biosystems) and with 0.3 µl GeneScan™ 600 LIZ™ Size Standard (Applied Biosystems) and with 1 µl of PCR product. Samples were run using the module G5, which performs an electrokinetic injection into the four capillaries for 16 s at 1,200 V. STR alleles were then separated at 15,000 V for approximately 20 min with a run temperature of 60°C.

Results

Experimental study

In our experimental study, 500 individual skin flakes were microscopically selected from tapings, extracted, quantified, and PCR-amplified. Results were reproducible for each system and also used different STR kits. For all samples, mixed DNA profiles could be avoided.

DNA profiles could be obtained from approximately 15% of all individually selected skin flakes (Fig. 1) using STR typing with increased cycle numbers in a LT-DNA (LT = low template) typing approach [7–9]. DNA quantification yielded highly variable DNA amounts within a wide range from ≤25 pg up to ≥200 pg/µl extract (Fig. 2). Approximately 15% of the single skin flakes could be successfully analyzed but more often yielded partial than complete STR profiles. Full DNA profiles could be obtained in approximately 5% of the samples (Fig. 3a, b). Further PCR

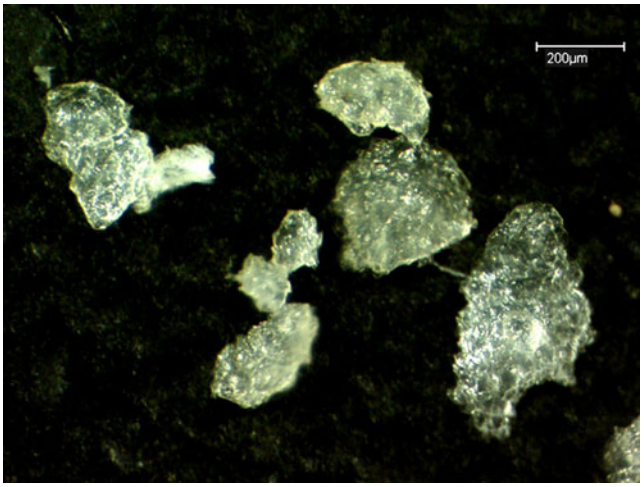

Fig. 1 Examples of single skin scales, which were selected by microscopic investigation. Magnification=100-fold

optimization using miniSTR protocols by generating smaller amplicons was more successful compared to commercial STR kits [4, 10, 11].

The microscopic selection of single skin flakes is a labor-intensive and time-consuming examination method, but has the potential to be extremely successful and can avoid mixed stain patterns.

In contrast to single skin flake analysis, if large areas of an item are swabbed or if large areas of material are taken for extraction purposes, all potentially transferred skin flakes from any number of individuals will be included in the extraction, thus resulting in a mixed DNA pattern, which in many cases, cannot be interpreted due to the complex nature of the mixture.

For typing of single skin flakes, a full profile can only be expected in 0.5–1% of selected scales using 30 PCR cycles and a boost to 33 cycles is necessary to increase the chance of obtaining a full profile. Such LT-DNA typing methods have higher risks of well-documented artifacts (e.g., locus drop-out, allelic drop-out and drop-in, stochastic effects, peak imbalance), and the results should be always confirmed by replicate analysis [12, 13]. Furthermore, miniSTR typing is more successful for LT-DNA samples

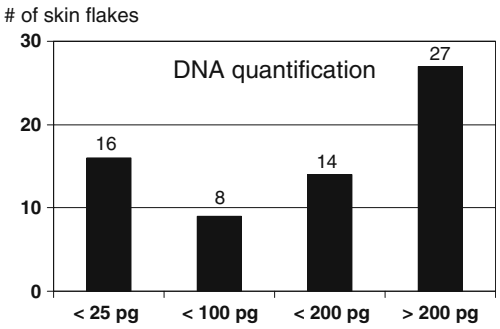

Fig. 2 Quantified amount of DNA (*N*=65 skin flakes)

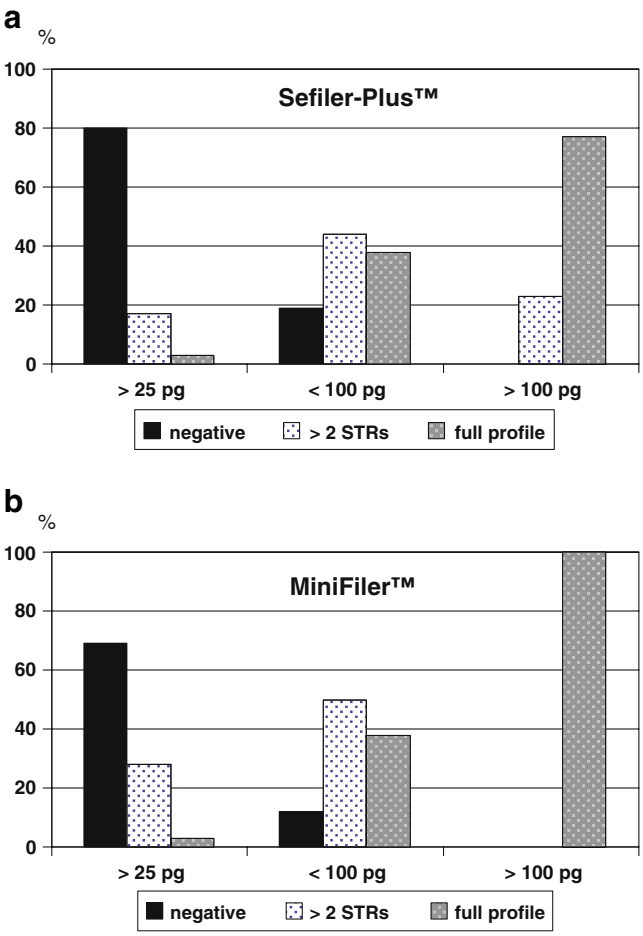

Fig. 3 Success rates of the two STR-kits (SEfiler, Minifiler) in correlation to the quantified amount of DNA. Threshold for allele calling 50 rfu. *N*=65 skin flakes. Values are given in percent

compared to standard STR kits because the sensitivity of STR typing can be increased substantially. The increased sensitivity of these methods requires that adequate engineering controls be implemented in the laboratory to preclude unnecessary trace intra-laboratory contamination.

Two casework examples are provided to demonstrate the usefulness of the microscopic skin flake selection strategy.

Case scenario 1

In October 2005, the 31-year-old Anita B. was found strangled and obviously sexually assaulted at a rest area on the interstate highway A45 near Cologne (Germany). In the early stages of the investigation, a standard sexual assault kit and the victim's clothes were submitted to a private forensic laboratory for DNA testing. After an examination lasting 4 months and a large number of DNA samples having been tested, no evidence relating to the offender was found, and the case was shelved and remained unsolved. In spring 2006, the case was reopened

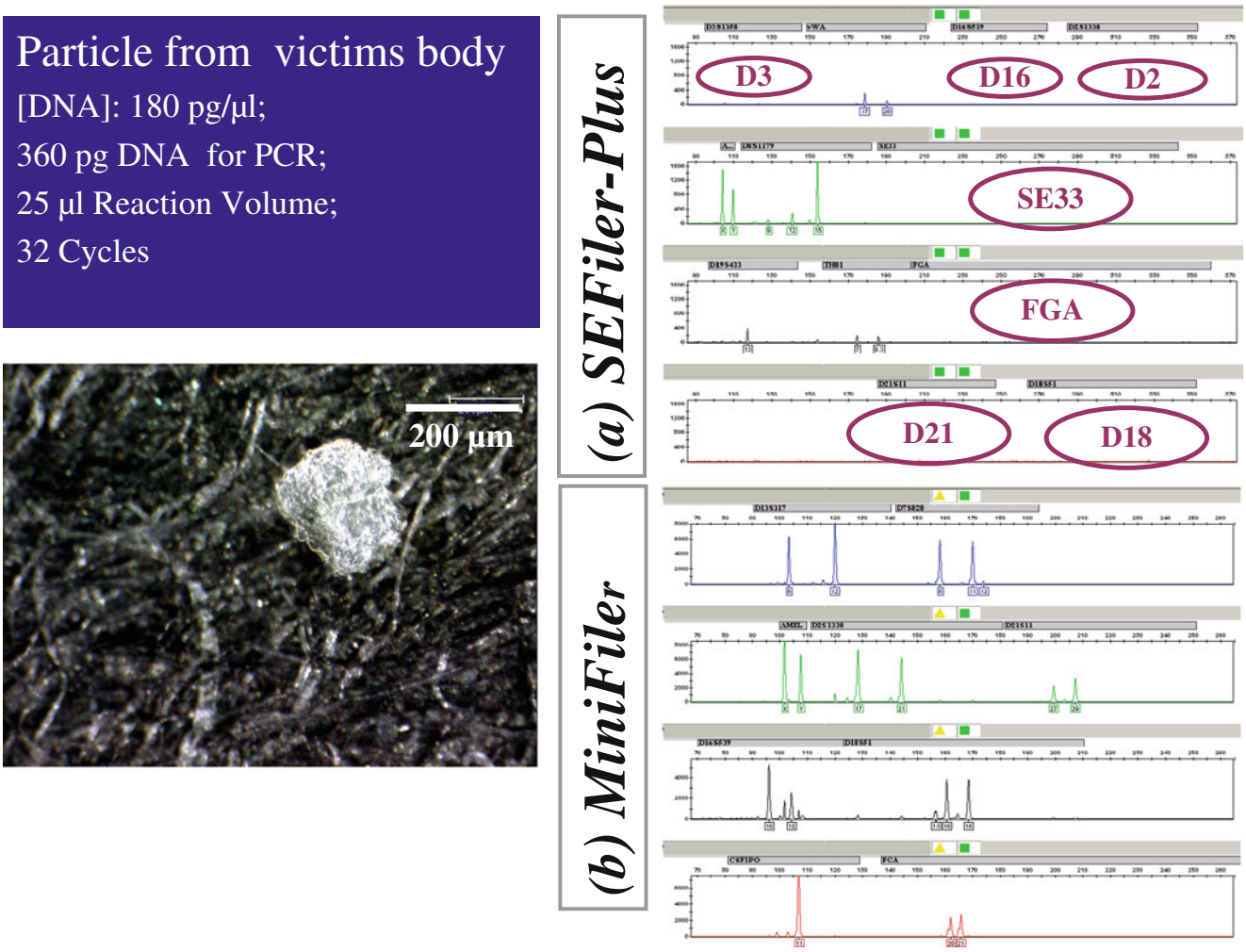

Fig. 4 **a** Initial SEfiler-plus profile obtained from one particle from the victim's body. **b** MiniFiler profile obtained from the same particle. Loci dropped-out are highlighted with circles

and all still existing specimens were reanalyzed. Although the most promising stains (e.g., intimate swabs, fingernail clippings) had been exhausted by the initial examination, a minute saliva stain could be detected on the victim's bra. Unfortunately, the DNA found in the sample was predominantly from the victim and only contained a very small male component. DNA investigations using standard autosomal and miniSTR systems in conjunction with Y-STR testing yielded a partial and low-level male profile, which was suitable for exclusion of all potential suspects but insufficient for a database search.

In July 2006, it was decided to make a further examination of the tapings from the victim's clothing. Using the preparation method described, approximately 200 skin flakes could be isolated from the victim's panties, and a full male DNA profile was found in 2 of the flakes, which matched the partial profile from the saliva stain on the victim's bra.

In August 2006, a search of the German DNA database (DAD) reported a match of the unknown male DNA profile with a profile found in two unsolved homicides and one attempted homicide, all committed on young female victims. This led to the conclusion that a serial killer was linked to all four crimes. By combining the information of these four cases and also by giving detailed information to the public, the police were able to identify a suspect in less than 2 weeks. At the end of August 2006, the suspect, a 29-year-old truck driver confessed to all the four cases. On June 4, 2007, after a 12-day court hearing, he was sentenced to life imprisonment without possibility of parole.

Case scenario 2

On 11 June 1989, 74-year-old Mary B. was found strangled and brutally abused in her flat in Frankfurt, Germany. During the initial crime scene investigation, detectives identified a piece of adhesive tape with a fragment of a disposable glove around the victim's neck, which had

obviously been left behind by the offender. The victim's body was comprehensively taped by the scene of crime officers for potential fiber analysis, but the tapes were never analyzed. For decades, the case remained unsolved but was finally closed in April 2009. Although some of the physical evidence had been lost or chemically modified by dactyloscopic examinations, the case was reopened and re-investigated in 2007. The adhesive tape from the victim's neck and the fiber tapings from the victim's body were analyzed. The tapings were microscopically searched, whereby approximately 400 potential skin flakes could be isolated from the tapings, which were individually subjected to DNA extraction and amplification as described.

Of the 400 skin flakes, 5 were found to contain a partial degraded male profile by using the standard autosomal STR typing procedures. By using the new miniSTRs, we were able to generate a full DNA profile (Fig. 4), which was entered into the national DNA database in December 2007. On August 8, 2008, a DNA cold hit notification was registered, which matched the profile of a 56-year-old bus driver, who had been recently convicted for a sexual offense committed in 2005. On August 13, 2009, the suspect was arrested and charged with the murder of Mary B. Without making a confession, he was sentenced to life imprisonment on April 30, 2009.

Discussion

The case examples presented here demonstrate that in the age of sophisticated DNA technology, the old fashioned method of microscope-aided manual searching can still play an important role in the identification of offenders and perpetrators of violent, and sometimes, even non-violent crimes.

As with most procedures for identifying contact between two persons, there are still difficulties and disadvantages which must be overcome. The rate at which transferred DNA will subsequently disappear is dependent on many highly variable, mostly environmental factors, which limit the use of this method in respect to transfer from the victim to the offender. Another confusing factor lies in Locard's principle itself as “every contact leaves a trace”, with the emphasis on “every”! Any form of contact between the victim of the crime and any person with whom contact has occurred over the previous 24 h can give rise to some degree of DNA transfer depending on the extent and force of the contact. As there is no means of assessing when DNA was transferred, any extraneous transfer not relating to the actual crime represents a “red herring” in the process of identifying an offender. However, under the circumstances of violent crime, it can be assumed that in most cases, the very last contact the victim had was with the offender, given that no after-the-crime contamination has occurred.

Transfer of DNA from the hands of the offender to the neck of the victim in cases of manual strangulation, as well as to the other parts of the body in cases of rape and murder, are the most obvious locations to begin searching for skin flakes, which have been transferred [3]. The anatomical location of DNA from the offender on the victim's body and the absence of transfer to other sites can be a decisive factor in establishing a causal link.

Given that the (dead) body of a victim usually remains in the same position until it is discovered, the disappearance of transferred DNA is reduced to a minimum, and theoretically at least, will remain undisturbed. This is a vastly different situation when compared to the rate of disappearance from a living victim, who is mobile and possibly undertakes measures of hygiene, such as a shower or bath, before any physical examination takes place.

A special aspect of this form of searching strategy is the investigation of cold cases. It has been the practice for many decades to tape the body of a victim, a victim's clothes, and sometimes even the surrounding areas, to preserve fibers for later investigations, if deemed necessary. This was not often the case, but even if the tapings had been examined for fibers these tapings were preserved and the DNA in the form of skin flakes should still be present, as only the fibers have been removed and can be used for a DNA search. Nobody ever imagined that these tapings, considered by some to be superfluous and time-wasting, could later be used for identifying DNA transfer from the offender.

Although the credibility of LT-DNA testing has been challenged recently and the arguments on its applicability in forensic casework are ongoing, there appears to be no doubt that such investigations can provide valuable information [14]. However, given the known artifacts which can occur with low numbers of template DNA, it is recommended that LT-DNA typing should be used primarily for investigative leads, and caution should be always taken when interpreting the results.

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