MULTIPLEXED DNA MARKERS FROM CIGARETTE BUTTS IN A FORENSIC CASEWORK

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Introduction

As described by Hochmeister et al. [1], suitable amounts of DNA for Polymerase Chain Reaction (PCR) may be recovered from saliva cells present on cigarette butts. In such cases, detection of DNA markers may be limited both by degradation and poor amount of extracted DNA. The analysis of STR loci by fluorescent-based technology [2-3] may reduce these limitations. Moreover, coamplification of different markers in the same reaction [2-3] allows to reach more informativity in defining identity of samples.

A case where 53 cigarette butts found in 3 different cars were analysed by a fluorescent-base technology after a multiplex reaction and compared to 10 suspects is here described.

Case report

A truck-driver was compelled to drive his truck to a suburban place by 2 masked people got off a car (Car 1) in a parking area. Other 2 masked people reached the truck and they all transferred goods from the truck to a pick-up driven by another person. The 2 first assailants were transported by the truck-driver to an area where got into a second car (Car 2).

The truck-driver quickly informed the police and a man (suspect 1) in his car (Car 3) was arrested. A gun was found in this car. The day after, other 9 persons were checked because found into possession of the stolen goods. 2 among them (suspects 3 and 4) were seen to get off the car of suspect 1 the day before. Cars 1 and 2 resulted stolen a few days before the action. A total amount of 53 cigarette butts were found in the 3 cars and in the truck, according to the scheme in Table 1.

Our laboratory was charged to employ DNA techniques with the aim to reach a positive identification comparing DNA from cigarette butts to the suspects.

A multiplex amplification of 4 STR markers (HUMvWA31/A, HUMTH01, HUMF13A1 and HUMFES/FPS) was performed. Gendering of cigarette butts was done as previously described [4].

Materials and methods

DNA extraction. A rapid Chelex extraction followed by Centricon 100 concentration was performed for cigarette butts. Phenol/chlorophorm protocols were used for fresh samples from suspects.
PCR conditions. Up to 10 ng from fresh samples and 10 µl of Chelex extracted material was amplified by a multiplex reaction as suggested by literature [2-3]. Amelogenin amplification was done according to Mannucci et al. [4].
Products analysis. From 1 to 5 µl of amplification mixtures were combined with an internal lane standard (GS2500), heat denatured and loaded onto a 6 % polyacrylamide denaturing gel. Electrophoresis was carried out for 5 hours and 30 minutes at constant power (36W) on an Applied Biosystems automated DNA sequencer 373A. Genescan 672 software (Applied Biosystems) was used to determine fragment sizes.

Results and discussion

In 50 samples out of 53 results were obtained both by multiplex reaction and gendering. The most of the samples (38) were referred to 4 suspects and 12 to 7 unknown men and 5 women (Table 1). In 3 cases no amplification products were obtained from cigarette butt extractions.

Referring to the 4 suspects, PCR products analyses allowed calculation of casual phenotype sharing respectively of $0.8x10^{-4}$ for suspect 1, 10^{-4} for suspect 2, $0.4x10^{-4}$ for suspect 3 and $0.3x10^{-5}$ for suspect 4.

As expected, the use of a multiplex reaction by a fluorescent-based technology gave useful indications for individual identification of suspects (Table 1).

Identity values by manual methods and silver staining would surely be lower than that reached by fluorescent-based techniques, because of the poor amounts of DNA recovered from cigarette butts. The use of a multiplex reaction allows detection of different markers using the same amount of extracted DNA that is commonly used for a single reaction. Moreover fluorescent-based technologies assure higher sensitivity and may disclose results unidentifiable by manual methods.

Conclusively, it can be stressed that such a technique is a powerful tool for forensic casework both for sensitivity and liability, when it is used as suggested by validated protocols.

Table 1. Number of cigarette butts per car referred to the identified suspects and unkown people. In 3 samples out of 53 no amplification products were observed.

	Car 1	Car 2	Car 3	Truck	Total
Suspect 1	2	2	18	1	23
Suspect 2	=	6	=	=	6
Suspect 3	=	=	2	=	2
Suspect 4	=	=	7	=	7
Unknown	=	1	11	=	12
No results	=	=	3	=	3
Total	2	9	41	1	53

References

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