International Congress Series 1261 (2004) 473–475

www.ics-elsevier.com

DNA typing from steel cable

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Abstract. The aim of this study is to analyze the possibility of DNA typing, on the specimen recovered at the crime scene. The optimization of extraction was carried out via an experimental system to reproduce the contact with the victim. The results obtained will be described in detail.

Keywords: Low copy number; Identifiler; PCR; Drop out

1. Introduction

We report a homicide attempt case which occurred in February 2003 in Sicily. The victim was assaulted with a steel cable put around the neck, but was able to run away, and the contact was only for a few moments. Police found a 3-m-long cable, which was 0.6 cm in diameter, at the suspect's house and immediately submitted it to our laboratory for the DNA typing. Results were immediately transmitted to the Public Prosecutor and the assailant was soon arrested.

Before the final analysis on the above specimen, we performed various types of experiments, according to LCN recommendation, to optimize the extraction phase and PCR DNA typing on different reference samples [1]. We set several experimental conditions to evaluate the minimum time of contact with the skin necessary to transfer sufficient quantities of biological material onto a steel cable similar to that described in the case report. DNA extraction and PCR conditions were optimized for low copy DNA typing on a different cable used as reference samples. Experimental time points were different. Sufficient DNA to perform the STR analysis was obtained from samples to complete DNA typing. The untreated cable, used as negative control, did not give any amplified product. Our result demonstrates that a brief contact of some objects with the human skin is sufficient to determine the transfer of some biological material and support the possibility to type DNA in a low copy number on specimens recovered at the crime scene.

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0531-5131/
doi:10.1016/S0531-5131(03)01715-1

2. Materials and methods

We utilized various kinds of steel cables, in order to better the reproduction of the original conditions of our case and to evaluate eventual differences in DNA quality and yield.

The specimen cables were at first cut into 10 bits and each bit was washed for 3 h in an ultrasonic bath in 1 ml of lysis buffer (10 mM Tris–HCl pH 8, 10 mM EDTA pH 8, 100 mM NaCl, 2% SDS, 40 mM DTT) in a single tube, with 15 ul di PK (20 mg/ml). The mix was vortexed at 1000 rpm to separate the lubricating grease. The surnatant was purified Microcon 100 Amicon following the manufacturer's recommendation. DNA extraction from the specimen and reference samples was carried out essentially by chelex procedure [2].

The amplification of the DNA extracted was performed by use of an *AmpFLSTR Identifiler kit* (Applied Biosystem), in 12.5 ul final volume. PCR followed in a Perkin Elmer Gene Amp PCR System cycler 2400 and 9700 [3]. According to the kit protocol, positive and negative controls were enclosed during the amplification step.

The product of reaction after 28 cycles was then analyzed and non-repurified with Microcon 100 Amicon and the analysis of amplified was performed following standard parameters on ABIPRISM 3100 DNA Sequencer Allele. Sizes were determined using Gene Scan v.2.1 software. Automatic allele designation was achieved by use of Genotype v.3.7 software (Applied Biosystems).

3. Results and discussion

The procedure we have described above, chosen after optimization on various kinds of steel cable, permitted the correct identification of the DNA extracted. Blood tests were negative and the DNA, perhaps, resulted from contact with the skin.

To obtain a suitable sample, we chose not to swab the cable surface, but to cut the steel cable into small pieces to treat with ultrasound in order to increase the extraction effectiveness and to decrease interference with potential contaminants (oil, dirt, etc.) the mix was vortexed at 1000 rpm to separate the lubricating grease.

The procedure utilized, which gave the maximum sensitivity, was as follows:

- Extraction using a strong buffer and sample sonication;
- Inhibitors removal through filtration;
- Single tube amplification with chelex;
- Reaction volume reduction;
- Post-amplification filtration;
- Electrophoresis on ABI 3100.

The experiment conducted with various steel cables in different experimental conditions permitted us to demonstrate that the 30-s contact between the cable and the human epidermis was sufficient to permit the extraction of amplifiable DNA (Fig. 1). Some artifacts were observed, such as; allelic drop out, but the influence on the interpretation was minimum.

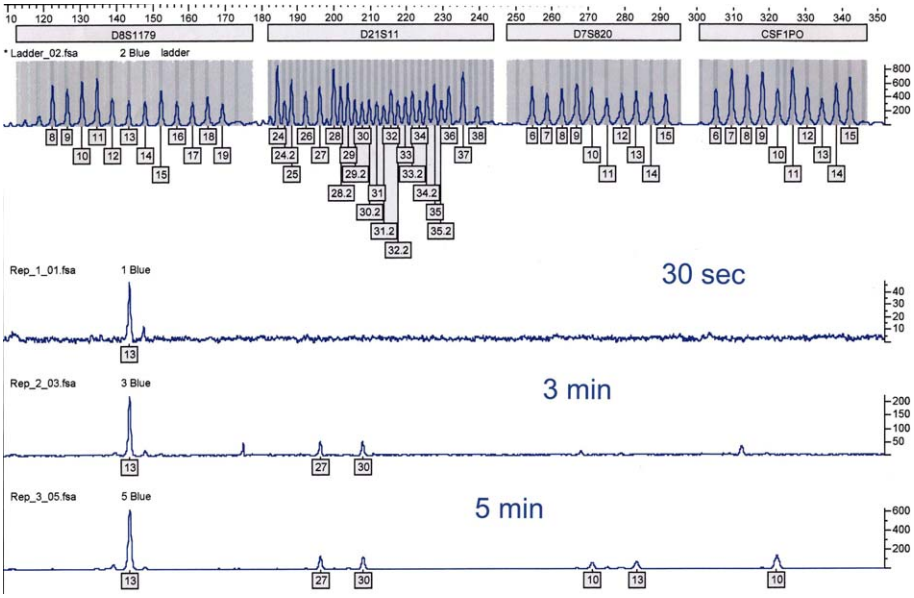

Fig. 1. DNA genotyping from different time of contact.

Conditions that can affect the amount of biological material transferred to the cable need to be experimentally evaluated; at this moment we are studying the influence of contact time on DNA recovery [4–6].

Acknowledgements

This research was supported by grant of Dr. R.A. Recupido and Dr. F. Sottosanti, Judge and Public Prosecutor of the Court of Catania.

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