

LMD as a forensic tool in a sexual assault casework: LCN DNA typing to identify the responsible

D. Di Martino ^a, G. Giuffrè ^b, N. Staiti ^a, A. Simone ^b, G. Sippelli ^b,
G. Tuccari ^b, L. Saravo ^{a,*}

^a *Laboratory of Molecular Biology, Raggruppamento Carabinieri Investigazioni Scientifiche (RaCIS),
Messina, Italy*

^b *Department of Human Pathology, University of Messina, Messina, Italy*

Abstract. We have previously studied the sensitivity of laser microdissection (LMD) techniques and have tested our capability to yield a complete genotype from 30 haploid sperm cells. Partial but significant genotype information have been obtained also from 5 to 10 haploid sperm cells. This experimental procedure has important applications both in pathological and in forensic fields. A real casework of a teenager sexually assaulted in a car in a small town in Sicily has induced us to evaluate the possibility to transfer the biological residuals present on a cut off from the car seat to a laser microdissection-prepared microscope slide. Biological traces have been at first examined with Crimescope CS-16 in order to evaluate the kind of traces we were to process; to avoid the loss of the forensic traces, we have reproduced the same conditions in several experimental procedures. We have settled several samples distinct for the amount of biological mixed traces, age of the traces and their exposure to different atmospheric agents. DNA typing was performed both with Identifiler STR loci kit and with another forensic kit based on shorter STR amplicons (MiniSTR). Laser microdissection techniques, coupled to high-sensitivity DNA typing methods as short STRs, allow forensic operators to isolate the different cell residuals whenever in front of mixed traces. © 2005 Published by Elsevier B.V.

Keywords: Low copy number; Laser microdissection; STR typing

1. Introduction

The outcomes of our previous researches performed with laser microdissector [1–3] have induced us to try to overcome the usual sensitivity limits of genetic investigations.

* Corresponding author. Tel.: +39 0905725687; fax: +39 0905725690.

E-mail address: rismebiologia@carabinieri.it (L. Saravo).

The typical main problems resulting from low copy number analyses may be the allele imbalance—up to the loss of an allele—within a genetic locus and PCR-generated slippage mutations, also called stutters [4,5]; dealing with single haploid cells, we had to solve other sort of problems mainly related to low copy number (LCN) DNA conditions like stochastic selection of each single spermatozoan [6] genotypic set. A real casework of a teenager sexually assaulted in a car in a small town in Sicily has induced us to evaluate the possibility to transfer the biological residuals present on the car seat to a laser microdissection-prepared microscope slide. Initially, biological traces have been scanned with Crimescope CS-16 in order to evaluate the kind of traces we were to process; to avoid the loss of the forensic traces, we have experimentally reproduced the same conditions in laboratory. The cells—spermatozoa and leucocytes—present in the mixed traces were correctly separated and singly STR typed, allowing us to distinguish genotypic profiles of the different donors whom biological matrix belonged to.

2. Materials and methods

2.1. Histological samples

Freshly collected seminal fluid and peripheral blood were earlier spotted onto the same filter paper and eluted with sterile physiological solution (0.95% NaCl in DEPC water). Then they were smeared on a microscope slide, covered with a special film properly embedding the microdissection samples and later Giemsa stained. Donors' blood was collected apart in order to obtain the complete genomic STR profiles to be compared.

2.2. Laser microdissection

A Leica AS LMD (Leica Microsystems, Germany) has been used to perform the isolation of small groups of cells, both spermatozoa and leucocytes: from any histological sample, we collected respectively 10 haploid and 5 diploid cells to extract DNA from.

2.3. Cell lysis, DNA extraction and quantitation

In order to obtain the best quality and sensitivity during DNA extraction, we have performed the method which already turned out to be the most sensitive: Chelex™ (Biorad) [7]; spermatozoa were incubated at 56 °C with 20 µg of Proteinase K and 0.1 M DTT for 1.5 h (leucocytes without DTT and for 1 h) then added with 3 µl 95% Chelex™, incubated at 56 °C for another 0.5 h and eventually boiled for 8 min. Extracted DNA was then centrifuged and freed from the Chelex solution. Donors' blood was extracted with 5% Chelex™ according to the manufacturer's protocol.

2.4. DNA amplification

DNA extracted from any sample (resuspended in 10 µl) was used for amplification as a whole and the traditional amplification hot start protocol was performed, according to the AmpFLSTR Identifiler PCR Amplification user's manual [8]. The number of amplifying cycles was risen up to 30 cycles.

2.5. STR typing

PCR products were separated on a polymer substrate by capillary electrophoresis in denaturing conditions with AB Prism 310 3100 Genetic Analyzers; results were analyzed by Genescan, Genotyper v3.7 and Genemapper software v3.2 (Applied Biosystems).

3. Results and discussion

The experimental procedure we set up has allowed us to solve a severe crime against a young innocent girl and, in the meantime, to face a scientific problem which is worthy to be shown: as a consequence of the low amounts of DNA sources investigators may find on crime scenes—but not as much as it happens when dealing with biological traces containing blood or any other kind of tissue made of diploid cells—when those sources contain haploid cells of a subject among the others, data interpretation turns out to be much more complex. In fact, in those circumstances allelic imbalance, typical of low copy number analyses, is due to the stochastic distribution of the single haploid cells in the substrate and the amplification effect usually known as “drop out” no longer affects these analyses as its own. The loss of an allele within a heterozygote couple is simply due to the absence of the cell carrying that chromosomal set, which might be easier to understand if related to the loss or imbalance of the gender-specific markers. We want to underline that statistical analysis of such an imbalanced genotype can be assumed for forensic judgment as well. The possibility of cross-contamination may still exist both on the crime scene and in the laboratory as some authors assess: that is why an LCN sample is always processed in different areas from where the routinely performed analyses are carried out. Attribution of a cell to a specific subject as an evidence may still be a problem if we refer to blood traces spread on the soil, but it is no longer a problem if we deal with spermatozoa coming from a vaginal swab!

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