

Molecular analysis of genomic low copy number DNA extracted from laser-microdissected cells

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Abstract. Tissue microdissection techniques offer the chance to characterize the several kinds of single cells present both in pathologic and in forensic samples cutting small tissue fragments as well as single cells by an ultraviolet laser beam under direct microscopic visualization. The low number of target cells among a wide spectrum of cell types in heterogeneous tissue samples may complicate the analysis of biological residuals found on crime scenes, while the possibility to perform a genomic analysis of low copy number DNA from few cells harvested by laser microdissection represents a valid aid to solve forensic problems. A complete genotypic profile was obtained down to 10 haploid and 5 diploid cells as to pathological and forensic genetic markers; different results—concerning the integrity of the extracted DNA—were achieved according to the kind of histological stainings performed. © 2005 Published by Elsevier B.V.

Keywords: Low copy number; Laser microdissection; STR typing

1. Introduction

Recently we have published a complete sequence of procedures we had performed in order to achieve the best results in DNA typing both according to pathologic and forensic needs [1–3]. Gill [4] and others have already reported some rules dealing with the interpretation of analytical results got from STR typing of an amount of DNA less than 100 pg. Diploid cells (lymphocytes), from 15 cells down to a single cell, were isolated by laser microdissection and then processed in order to extract and quantify DNA by Real Time PCR. DNA has been then STR typed through a 15 Loci multiplex PCR.

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2. Materials and methods

2.1. Laser microdissection

A Leica AS LMD (Leica Microsystems) has been used to perform the isolation of small groups of cells [1,2]: from any histological sample we collected respectively 15, 5, 2 diploid cells and a single diploid cell (Figs. 1 and 2) to extract DNA from.

2.2. DNA extraction, amplification and STR typing

Freshly collected human peripheral blood was earlier smeared on a microscope slide, covered with a special film properly embedding the microdissection samples, and later Giemsa stained. In order to obtain the best quality and sensitivity during DNA extraction we have performed several methods: A) Chelex™—cells were incubated at 56 °C with 20 µg of Proteinase K for an hour, then added with 3 µl 95% Chelex™ (Biorad), incubated at 56 °C for another half an hour and eventually boiled for 8 min. B) DNA IQ™ system extraction kit; C) Qiagen® DNA MicroKit—modifying the volumes of elution into microvolumes (< or =10 µl). The amount of extracted DNA from the 15 cells down to the single cell has been estimated by Real Time PCR, using an ABI Prism 7000 and Quantifiler Human kit produced by Applied Biosystems. DNA extracted from 15, 5 and 2 cells was divided into two aliquots and any couple of amplifications was lead both following the traditional amplification hot start protocol performed in our laboratory, and halving reaction volumes. DNA extracted from a single cell was amplified as a unique sample following the traditional amplification hot start protocol performed in our laboratory, according to the AmpFLSTR Identifiler PCR Amplification user's manual. In any case the number of amplifying cycles was arisen up to 30 cycles. PCR products were separated on a polymer substrate by capillary electrophoresis in denaturing conditions with AB Prism 310 and AB Prism 3100 Genetic Analyzers; results were, then, analysed by Genescan, Genotyper v3.7 and Genemapper softwares v3.2 (Applied Biosystems).

3. Results and discussion

In the present report we want to show some results achieved as a consequence of the laser microdissection of human peripheral blood smear (leucocytes). We have been able to extract low copy number DNA from 15, 5, 2 and 1 diploid cells with Chelex™—as Qiagen and IQ extraction protocols did not give any appreciable result—and to genotype it by the usual set of microsatellites we deal with in forensic caseworks for personal identification. Reported data are supported by the outcomes of Real Time PCR quantization procedures. Only has a single PCR parameter been modified during our experimental job: the number of amplifying cycles, which was established up to 30 in order to get a better quality of the genotypic profile at the same time without stutter or artificial drop-in products. The genotypic profile obtained since 15 cell samples was completely determined with all the 15 STR markers. Hence 5 and 2 cells were separately collected by laser microdissection in a single group and then processed. Extraction volumes were still reduced with respect to the previous experiments with 15 cells and the small aliquots were separately amplified in order to evaluate likely stochastic effects and drop-outs as a consequence of PCR reaction on such small quantities of DNA. Results show that both genotypic profiles were affected by stochastic variations in the distribution of allele peaks; nevertheless the latter ones



Fig. 1. Single cell selected on a slide.

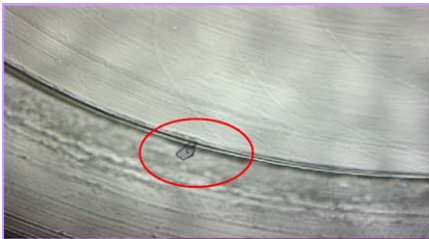


Fig. 2. LMD-based isolation of a single cell.

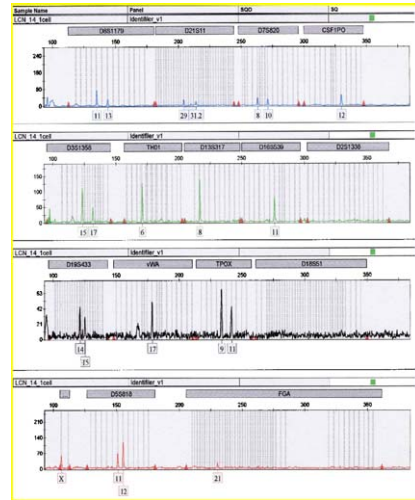


Fig. 3. STR profile from a single cell.

determined an almost complete profile useful for forensic comparisons. In fact, data interpretation coming from several amplifications of the same sample allows compensating for the above mentioned effects (drop-ins and drop-outs). DNA extraction from a single diploid cell was performed in a microvolume of 10 μ l, and included the volume of digestion and Chelex solution; the sample was amplified as a whole in 25 μ l of amplification volume and then STR typed on AB 3100 Genetic Analyzer in order to collect the total volume of extracted DNA. Anyway, to evaluate likely stochastic effects coming from the amplification of such a small amount of template DNA, we have performed several DNA extractions from single diploid cell samples at the same time. Results coming even from those experiments show we could get an almost complete genotypic profile (Fig. 3).

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References

- [1] D. Di Martino, et al., Single sperm cell isolation by laser microdissection, *Forensic Science International* 146S (2004) S151–S153.
- [2] D. Di Martino, et al., Laser microdissection and DNA typing from single hair follicles, *Forensic Science International* 146S (2004) S155–S157.
- [3] G. Giuffrè, et al., Analyses of genomic low copy number DNA from cells harvested by laser microdissection, *Pathologica* 96 (4) (2004) 396.
- [4] P. Gill, Application of low copy number DNA profiling, *Croatian Medical Journal* 42 (3) (2001) 229–232.