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A novel approach for genotyping of LCN-DNA recovered from highly degraded samples

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Abstract. Nowadays Short Tandem Repeats Polymorphisms are the most utilized genetic markers in forensic biology. A new kind of STRs profiling system is based on the amplification of shorter fragments compared to the conventional STR multiplexes; because of their length, these "Mini" STR amplicons could be obtained even from extremely degraded DNA and/or from few copies of template DNA. Mini STR kits for Human Identification have been already validated and placed on the market; moreover they are related to those loci which are commonly detected by traditional STRs kits, allowing comparison between data yielded with both methods. In this paper we report a homicide casework occurred in the South of Italy: a young guy was killed and then burnt. Nothing inside could be collected apart a burnt stub which was subjected to a comparative genotype analysis between STRs and MiniSTRs in order to verify efficiency and discrimination power of the latter ones. Our results show that MiniSTRs permit a reliable determination of 9 human allelic loci whereas traditional STRs typing protocols offer only a partial resulting genotyping profile. MiniSTRs turned out to be more robust and sensitive markers than the traditional ones. In the next future we aim to apply this method to several kinds of degraded samples in order to confirm the above mentioned observations. © 2006 Published by Elsevier B.V.

Keywords: Low Copy Number (LCN) DNA; STR typing; Forensic casework; Mentype®

1. Introduction

Despite a great power of discrimination (PD) and robustness, STR assays can be useless when highly degraded samples are found on crime scenes or in consequence of mass disasters; in these cases low DNA quality can significantly reduce PCR amplification efficiency, thus obstructing reliable genotyping. In order to overcome this drawback scientists initially turned to mitochondrial DNA (mtDNA) [1]; nevertheless, since mtDNA

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markers didn't result to be as discriminative as forensic STR kit ones [2], some authors tested the suitability of shorter STR amplicons (MiniSTRs) [3] for genotyping highly degraded DNA. Currently they are being increasingly employed for forensic purposes [3]. In this paper we report a casework that gave us the chance to evaluate a Mini STR assay for human identification from heavily spoilt samples. An individual was killed and his body was moved in an abandoned farmhouse. In order to erase every trace, the house was then burnt. Fire department officers intervened after a while by flooding the farmhouse and all the adjacent area with water. After the mud was removed, a deep on-the-spot investigation brought to light a smouldered cigarette butt which was submitted to authors' forensic laboratory for further analysis. Our aim was to compare STRs and MiniSTRs approaches in typing damaged genetic material adhered to the stub. Several difficulties were expected to arise since preliminary quantification by means of Real Time PCR, indicated an amount falling in the range of the so-called Low Copy Number (LCN) DNA. In order to reach our goal, we laid down an experimental protocol based on the Mentype® Nonaplex^{QS} kit (BioType AG), adjusted for specific thermocycling conditions.

2. Materials and methods

2.1. DNA extraction and quantification

DNA adhered to the stub was extracted through 5% Chelex™ (Biorad) in a final 50 μl volume, according to the protocol supplied by the manufacturer. Extracted DNA was purified by means of filtration on Microcon™ YM-100 and YM-30 filter device (Amicon, Millipore). The amount of extracted DNA was quantified by Real Time PCR, using an ABI Prism 7000 and Quantifiler Human kit produced by Applera Corporation.

2.2. DNA amplification and STR typing

After purification DNA extracted from the cigarette end was amplified, according to the AmpF*L*STR Identifiler and Mentype® Nonaplex^{QS} user's manuals. Each reaction was carried out by Gene Amp 9700 and 2400 thermalcyclers (Applied Biosystem). PCR products were separated by 310 AB Prism Genetic Analyzer; results analysis was performed by using the Genescan v. 3.7 software.

3. Results and discussion

DNA extraction and purification were successfully carried out by using respectively Chelex™ and Microcon™ YM-100 and YM-30. According to our experiences in genotyping LCN DNA from very few cells [4], this strategy resulted to be the most effective. Real Time PCR quantification tests indicated values ranging from 15 up to 30 pg/μl. As regards to DNA typing, the employed analytical approach permitted a meaningful comparison between traditional STR kit (Identifiler®) and MiniSTR kit (Nonaplex^{QS}). In particular Identifiler® kit produced incomplete profiles even though reiterated filtrations were attempted. The same approach was endeavoured with 30 and 32 amplification cycles; twenty-eight cycles programme still showed indisputably better results than the 30 and 32 cycle's ones. Notwithstanding genetic profiles were characterized by very low RFU values and still dramatically affected by stochastic variations like drop ins/drop outs and peaks imbalance. Hence we pursued the utilisation of Nonaplex^{QS} MiniSTR kit, strictly following

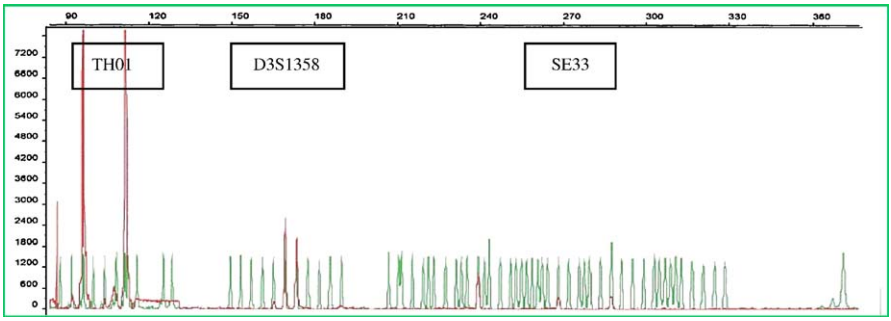

Fig. 1. MiniSTR profile obtained by 32 amplifying cycles programme.

a three-step experimental planning. Our first try concerned the simple use of MiniSTR kit, just following rigorously manufacturer's recommendations. Incomplete and useless data were gained, although a severe observance of the protocol had been pursued. At the second attempt we have substituted Jumpstart *Taq* Polymerase with *Taq* Gold® Polymerase so as to evaluate possible proficiency differences. Surprisingly not even a single allele was sufficiently amplified when *Taq* Gold® Polymerase had been added to the reaction mix. In the end we have striven to find optimal thermocycling conditions: small increments in the cycles' number dramatically improved amplification efficiency. A comparable 9 Loci profile was acquired by performing a 32 PCR cycles programme. MiniSTR-based profile overlapped with the partial STR genotype, confirming allele peaks obtained by Identifiler kit and improving power of discrimination (Fig. 1).

4. Conclusion

In cases of fire and mass fatalities the number of DNA copies really available as a template for PCR can be much lower than the total number of copies. That is to say that DNA quality and quantity cannot be evaluated independently. Initially in our experience yielded DNA wasn't usable for accurate genotyping. On the other hand such low DNA amounts as 15–30 pg/μl couldn't explain exhaustively total and partial amplification failures. Results of quantification and stochastic variations suggested indeed that DNA in these samples was so degraded that makes traditional STR kits ineffective. Employing technical adjustments developed for LCN DNA at the authors' laboratory, extracted DNA was subjected to innovative MiniSTRs profiling. Application of modified MiniSTR protocols within a LCN-based strategy permitted a satisfactory genotyping from few suitable DNA copies.

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