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Post-mortem DNA damage: A comparative study of STRs and SNPs typing efficiency in simulated forensic samples

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Abstract. DNA recovered at a crime scene often results as damaged; this represents enormous difficulty for the correct typing because of fragmentation or the lack of DNA region of interest. In this work a set of biological samples was prepared and stored under different conditions; STRs and SNPs typing was performed at regular interval of time in order to study the effects of natural DNA degradation. Allelic/locus drop-out phenomenon for the higher molecular weight loci or no results were obtained for microsatellite analysis after 1 week. SNPs typing gave positive results depending on storage conditions and type of substrate; a nucleotide alteration (C to T) was observed for M269 locus in a sample after 3 months.

Keywords: Short tandem repeat; Single nucleotide polymorphism; Post-mortem DNA damage

1. Introduction

It is well known that DNA is subject to enzymatic and chemical post-mortem damage [1]. DNasi I randomly cuts the histon-free double strand DNA and hydrolysis, oxidation, alkylation and cross-linking phenomena produce alterations at single nucleotides, hindering DNA polymerase activity or introducing mismatches [2,3].

This represents a source of errors for the correct typing in forensic casework: the STR markers commonly used give partial results with degraded DNA, and certain identification may be problematic. Forensic scientists have recently focused their attention on SNPs,

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which can be analysed in short amplicons, allowing positive DNA amplifications, even with degraded samples.

In published works, DNA degradation and its effects were examined using fragmented DNA by sonication and enzymatic treatment [4] but there have been no comparative monitoring studies on STRs and SNPs typing efficiency during natural degradation.

For this reason, a set of biological samples was prepared and stored under different conditions and STRs and SNPs typing done at regular intervals.

2. Materials and methods

2.1. Sample collection

20 g of male muscle tissue stored in the open air (A), buried (B), immersed in river (R) and sea (S) water, and dry (D) and wet (W) bloodstains, was prepared. Fresh blood and muscle tissue of the same subject were collected for a reference sample.

DNA was analysed after 1 week, 2 weeks and then monthly for 9 months, for a total of 11 samplings.

2.2. DNA extraction and quantification

DNA was extracted by phenol–chloroform and submitted to agarose gel electrophoresis and PAGE in order to check the degree of fragmentation. All samples were quantified by QuantiBlot Human DNA Quantitation Kit (AB) using a probe with a sensitive range from 2 ng/μl to 0.016 ng/μl.

2.3. STRs and SNPs typing

DNA samples were typed by AmpFlSTR Identifiler PCR Amplification kit (AB), and by 6 Y-SNPs PCR multiplexes developed in our laboratory [5]. STRs were amplified according to the manufacturer in a final volume of 25 μl. Y-SNP amplifications were performed using Hot-start Taq polymerase (Qiagen) in a final volume of 12.5 μl. After enzymatic purification, 1 μl of PCR product was submitted to single base extension using the SNaPshot Multiplex Kit (AB) in a final volume of 5 μl. Both amplification and extension products were run on an ABI Prism 310 (AB) and analysed by Genescan Analysis 3.7 (AB) software.

3. Results and discussion

DNA extracted from muscle tissue samples submitted to agarose gel electrophoresis and PAGE showed a smeared pattern after 1 week, while bloodstains gave negligible or negative results.

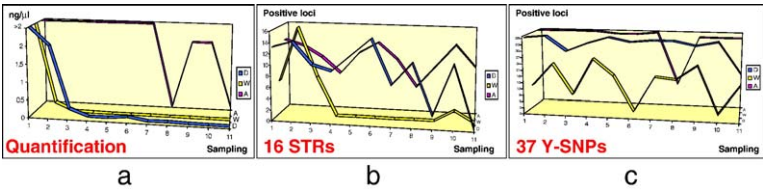

Fig. 1. Quantification and amplification trends for dry (D) and wet (W) bloodstains and muscle tissue stored in the open air (A).

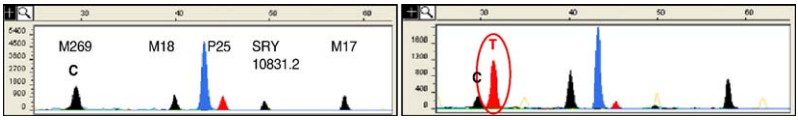

Fig. 2. Nucleotide alteration (C to T) observed at the SNP locus M269 in wet bloodstain DNA typing after 3 months of treatment.

Quantitation analysis revealed no human DNA in muscle tissues immersed in river and sea water after 1 week and in buried muscle after the first month. In the remaining DNA specimens – dry and wet bloodstains, muscle stored in open air – a progressive decrease of detectable human DNA was observed (Fig. 1a), later on (after the fifth month) in muscle tissue stored in open air.

STRs typing showed negative results from the second week in buried muscle tissues and immersed in river and sea water, in accordance with the Quantiblot results; allelic and locus drop-out phenomena were observed in all other samples (dry and wet bloodstains and muscle tissue stored in the open air) from the first week (Fig. 1b).

SNPs typing gave positive results in muscle tissue stored in the open air and in dry bloodstains at all 11 samplings, whereas discontinuous positive results were obtained in wet bloodstains (Fig. 1c). A significant decrease in the number of positive SNPs loci was observed for muscle tissue stored in the open air at the eighth sampling (6 months); this may be connected to a man-made DNA extraction error as suggested by the quantification results (Fig. 1a). All other specimens – muscle tissue buried and immersed in river and sea water – gave negative Y-SNPs profiles after the second week. Amplification reactions of buried samples produced negative results despite a positive DNA quantification (ranging from 0.125 ng/μl to 0.016 ng/μl). In order to remove or minimize possible inhibitory effects, PCR reactions using 1:10 diluted DNA as template were performed, but no results were obtained.

The best results were achieved in muscle tissue stored in the open air for both human DNA quantification and amplification; a good profile was also observed with dry bloodstains. The worst results were obtained with wet bloodstains: this could be explained by the rapid and massive DNA degradation caused by a wet environment [3].

A base alteration (C to T) was observed at locus M269 in the wet bloodstain at the third month, Fig. 2); this phenomenon may be caused by enzymatic and chemical post-mortem damage [6].

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