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Capillary electrophoresis reveals DNA damage in aged forensic samples

P. Fattorini[a,*], F. Tomasella[a], E. Albertini[a], P. Grignani[b], C. Previderè[c]

[a] *Department of Sciences of Public Health, University of Trieste, Via molino a vento 123, 34137 Trieste, Italy*
[b] *IRCCS Policlinico S. Matteo, Department of Legal Medicine Pavia, Italy*
[c] *Department of Legal Medicine and Public Health, University of Pavia, Italy*

Abstract. Capillary electrophoresis was used to analyse aged forensic DNA samples. A good correlation between DNA damage and reliability of PCR typing results was found.

Keywords: Capillary electrophoresis; DNA damage; PCR fidelity

1. Introduction

PCR fidelity is influenced by several factors with the integrity of the primary structure of the template playing a major role [1,2]. DNA damage, studied by reversed-phase HPLC/MS, showed that aged forensic samples exhibited reduced amounts of the four canonical DNA bases and the presence of several molecular products of DNA oxidation [3]. Capillary electrophoresis (CE) has shown to be a powerful tool to identify DNA bases and their analogous [4]. In the present work, we used this technical approach to analyse aged forensic samples. In addition, to investigate a correlation between DNA damage and the reliability of the PCR typing results, we analysed the DNA extracted from several different forensic specimens.

2. Materials and methods

In order to evaluate the robustness of CE, 200 nM of each DNA bases (C, G, A and T) and 5-BrU (employed as internal standard) were dissolved in 1% HCl and injected by pressure (10 s) in an uncoated fused silica capillary (length 57 cm, ϕ 50 μm). A P/ACE 5000 (Beckman) apparatus was employed and the detection of the effluents was performed at 254 nm. The raw data were analysed by the System Gold

* Corresponding author. Tel./fax: +39-040-946153.
E-mail address: fattorin@univ.trieste.it (P. Fattorini).

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Table 1
Forensic samples analysed in this study

Sample	DNA source	Age	uDNA bases	PCR results
PV1A*	B	0.5	0.43	–
PV1B*	B	0.5	0.53	–
PV1C*	B	0.5	0.64	–
PV2A^	B	20	0.45	–
PV2B^	B	20	0.39	–
PV3A§	B	5	0.61	–
PV3B§	B	5	0.64	–
PV4	B	50	0.20	–
PV5	BS	0.5	0.31	–
TS1	BS	13	0.64	–
TS2	BS	12	0.43	–
TS146A	M	5	0.56	±
TS367D#	M	33	0.23	–
TS367F#	C	33	0.58	–
TS26	C	5	0.26	–
TS133A	C	5	0.84	+
TS2188	B	3	0.36	–
TS2189	B	2	0.87	+
CTRL	blood	–	>0.95	+

DNA source refers to the biological tissue from which DNA was extracted: B: bone, BS: bloodstain, M: muscle, C: cartilage. Age refers to the presumptive post-mortem time (given in years). uDNA bases refers to the relative amounts of undamaged DNA bases [3] found in the samples. PCR results refers to the reliability of the multiple PCR typing determinations: + reproducible results; – no genetic profiles; ± presence of PCR artefacts. Marks *, ^, § and # refer to samples collected from the same biological donor.

(Beckman). Replicate tests of this standard solution showed an error standard deviation $<2\%$.

DNA extraction was performed following standard methods [3]. About 1 g (estimated by EtBr staining) [5] of the DNA samples listed in Table 1 was hydrolysed in 90% formic acid at 170 °C for 30 min. After lyophilisation, the samples were redissolved in 25 μl of 1% HCl containing 25 nM of 5-BrU and analysed as reported above. As controls, eight DNA samples extracted from fresh blood were employed.

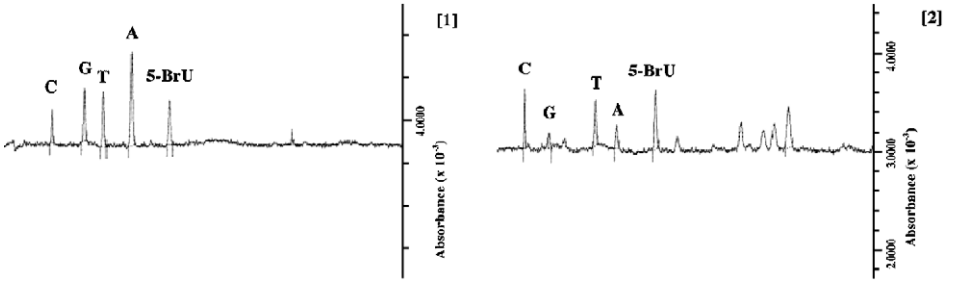

Fig. 1. CE analysis of a control sample extracted from fresh blood (chromatogram 1) and forensic sample PV1A (chromatogram 2). C: cytosine, G: guanine, T: thymine, A: adenine, 5-BrU: 5-bromouracil (internal standard control). Lower relative amounts of the four canonical bases and additional peaks are shown.

Comparable amounts (from 100 to 5 ng) of each forensic sample were amplified using the AmpF/STR *Identifiler* commercial kit (PE Applied Biosystem). Thirty cycles of PCR were performed. The PCR products were analysed by CE using an ABI Prism 310 Genetic analyser (PE Applied Biosystem).

3. Results

CE analysis of the forensic samples showed low relative amounts of undamaged DNA bases [3] compared to control samples (see Table 1). In addition, also the presence of several additional peaks was observed (see Fig. 1).

Replicate PCR amplifications provided reliable results only from samples TS133A and TS2189, while, in sample 146A, several PCR artefacts, such as allele "drop-out" and "drop-in", were found. No PCR products were obtained from the remaining samples tested in this study.

4. Discussion

In this work, we provide our experience on the employment of CE to analyse the integrity of the primary structure of the DNA extracted from aged forensic samples.

Our preliminary data confirm that CE is an accurate and sensitive method to detect chemical modification of the DNA recovered from aged forensic samples. The sensitivity of our method allows a powerful evaluation of the chemical integrity of less than 1 g of genetic material. Data from Table 1 strongly suggest that reliable STR typing can be achieved only from templates whose chemical composition is preserved. This is confirmed by other experimental data showing that both the processivity and the fidelity of *Taq* polymerase are influenced by the chemical integrity of the template [2,3,5,6].

In conclusion, our results show that CE is a powerful tool to investigate the molecular decay of forensic samples.

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