International Congress Series 1288 (2006) 522–525

www.ics-elsevier.com

Application of mini-STR loci to severely degraded casework samples

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Abstract. Four PCR-multiplexes of mini-STR loci have been used to get a nuclear DNA profile from different severely degraded casework biological specimens that generated negative PCR results or partial profiles when commercial STR kits were employed. Mini-STR technology allowed to retrieve additional genetic information with very high efficiency especially for those STR loci with allele sizes less than 150 bp. Our data indicate that the mini-STR technology is an effective strategy to improve DNA profiling from severely degraded casework human DNA samples that are refractory to amplification of DNA fragments bigger than 200–300 bp.

Keywords: PCR; STR; Mini-STR; Degraded DNA

1. Introduction

The most common type of DNA damage of forensic and ancient human remains is its degradation to small size fragments as a consequence of both endogenous and exogenous nuclease activity and non-enzymatic hydrolytic cleavage generating strand breaks. Here we evaluate the effectiveness of mini-STR multiplexes recently developed by NIST [1–4] to perform DNA profiling from severely degraded casework samples.

2. Materials and methods

2.1. Markers analyzed

Four PCR-multiplexes of mini-STR loci [1,2]: (Big Mini multiplex, Miniplex 5, Miniplex NC01 and Miniplex NC02) (see Table 1) have been used to get a nuclear DNA

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0531-5131/
doi:10.1016/j.ics.2005.10.044

profile from different severely degraded casework biological specimens that generated negative PCR results or partial profiles with commercial STR kits (Identifiler and PowerPlex 16).

2.2. Samples analyzed

The following forensic samples were extracted, quantified by real-time PCR [5] and amplified for all PCR-multiplexes of mini-STR loci: 2 soft tissue fragments fixed and stored in formalin for 3 years, 3 exhumed remains (two teeth and one compact bone) from a formalin-embalmed cadaver, 7 formalin fixed and paraffin embedded biopsies and 16 old bone remains from mass graves of the Spanish Civil war (1936–1939).

2.3. PCR amplification

Amplification of all multiplexes were performed according with original protocols described by Butler et al. [1] and by Coble and Butler [2], except annealing temperature for the Miniplex 5 and NC01 that were increased to 61 °C.

2.4. Analysis on ABI 310

Residual dye molecules were removed by washing products of amplification with TE 1× using Centricon 100. The amplification products were separated electrophoretically using an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with filter set F and four dyes 6-FAM, VIC, NED and ROX, with appropriate matrix.

3. Results and discussion

In all cases, mini-STR technology allowed to retrieve additional genetic information with very high efficiency especially for those STR loci with allele sizes less than 150 bp. However, in some

Table 1
Information on 15 STRs employed in this study

STR locus	Codis	Big Mini	Miniplex 5	Miniplex NC01	Miniplex NC02	Allele range	Size (bp)
TH01	Yes	6-FAM (B)				3–14	51–98
FGA	Yes	6-FAM (B)				12.2–51.2	125–281
CSF1PO	Yes	VIC (G)				6–16	89–129
D21S11	Yes	VIC (G)				24–38.2	153–211
TPOX	Yes	NED (Y)				5–14	65–101
D7S820	Yes	NED (Y)				5–15	136–176
Penta D	No		6-FAM (B)			2.2–17	94–167
Penta E	No		VIC (G)			5–24	80–175
D2S1338	No		NED (Y)			15–28	90–142
D10S1248	No			6-FAM (B)		10–20	83–123
D14S1434	No			VIC (G)		13–20	70–98
D22S1045	No			NED (Y)		5–16	76–109
D1S1677	No				NED (Y)	9–18	81–117
D2S441	No				VIC (G)	9–17	78–110
D4S2364	No				6-FAM (B)	8–12	67–83

All markers are tetra-nucleotides, except the trinucleotide D22S1045 marker. B: blue, G: green, and Y: yellow.

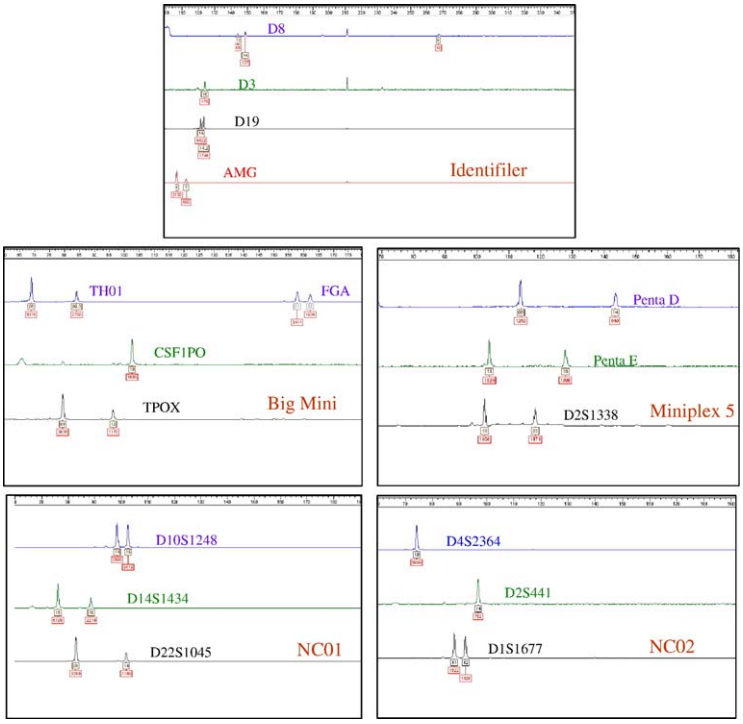

Fig. 1. Identifiler, Big Mini, Miniplex 5, miniplex NC01 and miniplex NC02 results for a nose fragment fixed and stored for 3 years in formalin. Peak labels are allele calls and peak heights in relative fluorescence units. No amplification products were obtained for D21S11 and D7S820 loci with the Big Mini multiplex.

cases, due to the high degradation degree, an extremely peak imbalance was observed between the smaller (60–100 bp) and bigger-sized (120–170 bp) STR markers or even between the smaller and the bigger-sized alleles of the same STR in heterozygote samples (see Fig. 1). Therefore, in some cases singleplex-PCR amplifications were carried out using different amounts of DNA template and different PCR cycles (28–32 cycles) to improve the quality of STR profiles. On the other hand, different artefactual peaks were observed that were removed by filtration of PCR reactions with Centricon centrifugal devices.

In conclusion, our data indicate that the mini-STR multiplexes offer an effective tool for recovering information in degraded forensic samples that generated negative results or partial profiles with commercial STR kits.

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