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Y-SNP typing by SNaPshot™ in the Belgian population and in degraded forensic samples

N. Siebert, B. Hoste*

National Institute of Forensic Sciences, Ch. Vilvorde 98-100, B-1120 Brussels, Belgium

Abstract. Eleven Y-SNPs were typed by minisequencing in a sample of 54 persons of the Belgian population. Seven haplogroups, according to the Y-Consortium nomenclature 2002, were observed with the following frequencies: 7.4% E-M35*, 24.1% F-M89* (× M9), 3.7% K-M9* (× 92R7), 1.8% P-92R7* (× M17, P25), 57.5% R-P25* (× M18, M153, SRY-2627), 3.7% R-SRY-1532b-M17, 1.8% R-SRY2627. In a series of very degraded post-mortem samples from seven victims, four could not be typed for STRs. The Y-SNPs, in 55–87-bp amplicons, were more successful showing full profile for two of these four samples, but no result for the two others.

Keywords: Y chromosome; Single nucleotide polymorphism; Minisequencing; Population; Degraded DNA

1. Introduction

Single nucleotide polymorphisms (SNPs) are a promising tool for very degraded forensic samples, as they can be analysed in very small DNA fragments. We started to test this application with Y-SNPs. After a blind inter-laboratory exercise of the EDNAP group, our SNaPshot technique for 11 Y-SNPs was applied to a Belgian population sample. Seven of the 11 Y-SNPs, in DNA fragments of 55–131 bases, were compared to STR (106–342 bases) in very degraded post-mortem samples.

2. Material and methods

Unrelated male persons of Belgian origin from all over the country. Post-mortem samples from seven male victims, stored at − 70 °C, an aliquot thawed twice was left at room temperature for 3 weeks. *DNA isolation*: proteinaseK digestion, phenol–chloroform and Centricon-30 (Millipore) purification. Teeth were first crushed in liquid nitrogen. *DNA quantification*: Quantiblot kit (Applied Biosystems). *STR typing*: in 28 cycles with the ProfilerPlus kit (Applied). *First PCR*: the 11 Y-SNPs were amplified in 3 multiplexes (Table 1). Based on the results of *3-plexI*, either *3plexII* or *4-plexI* was typed to determine

* Corresponding author. Tel.: +32-22434615; fax: +32-22416105.
E-mail address: Bernadette.Hoste@just.fgov.be (B. Hoste).

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Table 1
PCR and minisequencing (MS) primers for 10 Y-SNPs

Multiplex	Locus	Amplicon size (bp)	MS size (b)	MS strand	Mutation (F-strand)
3-plex-I	SRY-1532	87	36	R	A>G
					G>A
	92R7	55[a]	32	F	G>A
	P25	77	57	R	C>A
3-plex-II	M17 (M18)	70	44	F	+> – G, i.e. G>T
	M153	74	47	F	T>A
	SRY-2627	131[a]	62	F	C>T
4-plex-I	M89	73	24	R	C>T
	M9	341[b]	40	R	C>G
	SRY-8299	218	29	F	G>A
	M35	86	48	R	G>C

bp: base pairs, b: bases, F: forward, R: reverse. M17 and M18 on the same amplicon. All other primers are new, chosen with the Primer3.0 program v.0.2 (www.genome.wi.mit.edu/cgi-bin/primer/primer3), except [a] according to Ref. [2], [b] according to Ref. [1]. The length of the SNaPshot primers was adjusted with tails of non human sequence [3].

the haplogroup. The 2–5-ng DNA template in a 25-μl reaction mix containing 1 × PCR Gold buffer (Applied), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer except 92R7 (0.1 μM) and 2 units Taq Gold DNA polymerase. For the tetraplex *4-plexI* or for a hexaplex of *3-plexI* and *3-plexII*, the *Multiplex PCR mastermix* (Qiagen) was used. All multiplexes were run with 11 min 95 °C, then 30 cycles of 30 s 94 °C, 90 s 58 °C, 90 s 72 °C. Only for the optimisation, the forward primers were fluorescently 5′ labelled. After Microcon-30 (Millipore) purification, the PCR product sizes (in bp) were analysed on a 377 ABI-Prism sequencer. M18 (± 2 bp) was typed at this step. M17 (± 1 bp) on the same fragment was typed in the next step as the other Y-SNPs. *Minisequencing*: with the SNaPshot kit (Applied). After CentriSep (Applied) cleaning, the sizes of the elongated primers were analysed on the 377 sequencer, with the SNaPshot positive control as external size standard. The PCR primers (Applied) and the minisequencing primers (Qiagen) are listed in Table 1 (sequences available on request).

3. Results

Negative controls, i.e. female DNA (5 ng), blancos (all reagents except DNA) for DNA extraction, first PCR and SNaPshot, as well as PCR products in the SNaPshot mix without the SNaPshot primers, showed no peaks in the Y-SNPs range. In part of the male samples, double peaks were observed for 92R7 G/A or A (Fig. 1, row 1) and P25 C or C/A. They are due to locus duplication [2,4]. The detection threshold was 250 pg, with a 30-cycle PCR.

Y-SNPs haplogroup frequencies are given in the abstract [5]. These frequencies are close to the data in another sample of the Belgian population typed by restriction for a slightly different set of Y-SNPs [6].

Both methods were tested on the same amount (1–2 ng) of very degraded DNA. Victims 1, 3 and 4 showed full STR-profile. Victim 7 (whole canine) only showed a very partial STR-profile (Fig. 1). The STR-profile of drowned victim 2 (testis) was even

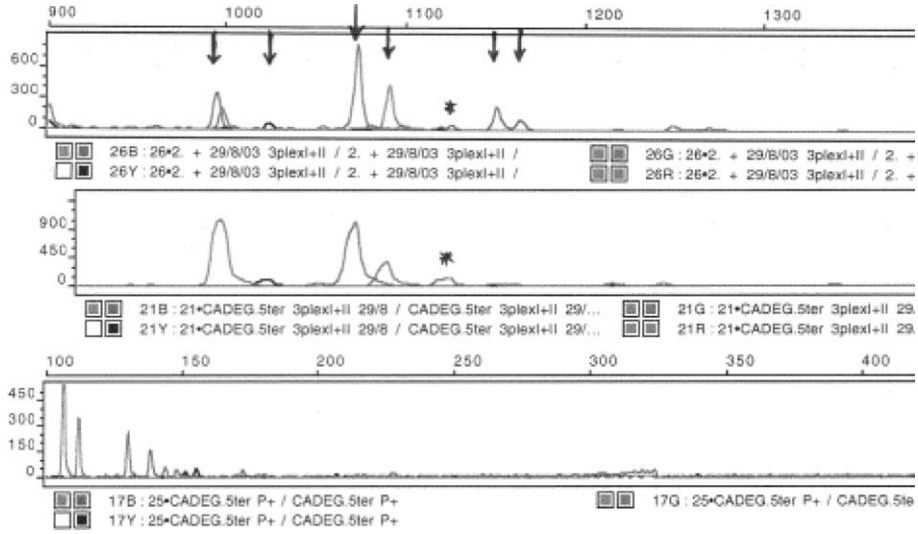

Fig. 1. Y-SNP and STR profile in a very degraded post-mortem sample and in high quality DNA. Row 1: Y-SNPs in high molecular weight DNA. Row 2: Y-SNP in degraded DNA (victim 7). Row 3: STRs (ProfilerPlus) for the same degraded sample. →: The Y-SNPs are 92R7, SRY1532, M17, M153, P25, SRY-2627 in increasing size (P25 lacking in row 2 due to a problem of primer conservation). *: Fluorescent first PCR product 92R7 55 bp.

weaker, as only 0.2 ng were amplified, due to the presence of inhibitors. All samples showed full Y-SNP profiles with fragments up to 87 bp, although the 131-bp fragment could be missing. Victim 5 (a blood sample taken several weeks after death) and victim 6 (whole canine of an old man buried for 7 years) yielded no result by either method. Their DNA amounts were under the detection threshold.

4. Conclusion

We have developed a multiplex of Y-SNPs. Our preliminary results in a small series of seven very degraded post-mortem samples show that, as expected, SNPs in 55–87-bp amplicons are more successful than STRs. Nevertheless, it seems from this limited series that two scenarios often show up: either the degradation is weak allowing the typing of STRs *and* Y-SNPs (if enough DNA is available), or the degradation is so strong that *neither* STR *nor* Y-SNPs can be detected. Further work needs to be done to improve the technique and to refine the results.

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