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Dealing with low amounts of degraded DNA: Evaluation of SNP typing of challenging forensic samples by using massive parallel sequencing

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ABSTRACT

A set of eighty-two forensic samples with different levels of degradation, as well five *in vitro* damaged samples were analyzed by the Precision ID Identity Panel. PCR amplifications were performed with scalar amount of DNA (from 1 ng to 12 pg) and through different number of cycles. A minimum coverage of 50 x was adopted for "locus call". Very informative profiles (based on about 65–70% of the loci) were obtained even in highly degraded samples when the amount of template range from 0.1 to 1.0 ng. When dealing with low amount of degraded DNAs, no more than half of the loci were typed, and the risk of mistyping (due to drop out phenomena) increased dramatically. The employment of a high number of PCR cycles is discussed.

1. Introduction

MPS technology provides advantages for forensic analysis due to its capability to genotype a large number of markers in a single assay. One of the most important challenges in forensic casework is to get a DNA profile suitable for identification starting from low amount of degraded DNA, which often returned an incomplete STRs profile. It is well known that SNPs markers display high successful rate with degraded DNA samples due to short amplicons length, and therefore SNPs typing protocols have been developed for forensic application [1–5]. Several SNP typing methods are available, each one with advantages and limits; moreover, in the last years the potential of MPS technology in SNPs typing was also largely investigated [1–5]. Thermo Fisher released a commercial multiplex SNPs panel targeted to individual discrimination. The Precision ID Identity Panel is designed to detect 90 autosomal SNPs together with 34 Y-SNPs by using massive parallel sequencing (MPS) technology. This panel has been optimized for forensic samples as it ideally requires 1 ng of DNA as input, but suitable profiles have been obtained even using input quantities as low as 0.1 ng [2–5].

To improve our understanding over the performance of this panel in casework analysis, a collaborative exercise involving six Italian forensic genetic laboratories was set up. The aims of the present study were i) to investigate the effectiveness of the Precision ID Identity Panel with low amounts of degraded samples; ii) to optimize the analytical conditions for this kind of samples.

2. Material studied, methods, techniques

Six laboratories collected a set of forensic samples (n = 82) including saliva swabs, bone remains, cadaveric blood, cadaveric muscle, fingernails, FFPE tissues, touch DNA, together with a set of artificially degraded DNAs (n = 5). The availability of a reference sample (either "same donor" sample or "first degree relative" sample) was an essential recruitment criterion.

DNA concentrations were assessed by duplicated tests in all samples by using the Quantifiler™ Trio DNA Quantification Kit. Libraries for MPS assay were prepared by using the Precision ID Library Kit according to the user guide [6]. Amplifications were performed with DNA input ranging from 1 ng to 12 pg and through different number of PCR cycles (from 21 to 26). For sensitivity studies, the 2800M DNA was used

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Table 1
Number of samples which gave full profiles (*e.g.* all loci with at least 50 x of coverage) according to the degradation index intervals (first column) and the DNA input range (first row) The data (n = 22) of the control DNA sample (2800 M) were pooled with those of samples with degradation index (D.I.) < 5. n.d.a.: no data available.

group	#1 (0.1–1.0 ng)	#2 (13–99 pg)	#3 (12 pg)
D.I. < 5	47/56	8/19	0/14
D.I. > 9	1/14	0/28	0/28
D.I. n.m.	0/21	n.d.a.	0/23

from 1 ng to 12 pg. Negative amplification controls were added for each round of PCR. MPS assays were performed by the Ion Torrent PGM platform and 256 barcoded libraries were sequenced on four chips. Individual SNPs genotypes were called by the HID SNP Genotyper plugin on the Torrent Suite 5.0.4. A minimum coverage of 50x was adopted for a "locus call" [7]. The relative Depth of Coverage (rDoC) across all loci sequence was calculated as the ratio of Depth of Coverage (DoC) at single locus to total DoC of the sample. For any data elaboration, the samples were pooled in three groups that were named, according to the DNA input used for PCR, as follows: #1: 0.1–1.0 ng, #2: 13–99 pg and #3:12 pg, respectively (see Table 1).

3. Results and discussion

The Quantifiler™ Trio DNA Quantification Kit showed the presence of DNA in all the samples. The degradation index (DI), showed no or low DNA degradation (DI < 5) in 69% of the cases, high degradation (DI > 9) in 7% of the samples, while the DI was not measurable (n.m.) in 24% of the samples due to the failure to amplify or to a very low amplification result (< 0.001 ng/μL) of the large amplicon.

The rDoC was assessed in five samples with different levels of degradation, which were amplified in duplicate by using 1 ng of input DNA. A uniform rDoC distribution across all 124 loci in control samples, regardless of the amplicons' size, was found. On the contrary, we observed a decrease of the rDoC values at the high molecular weight loci in samples with higher DIs.

Full profiles (*e.g.* all loci with a coverage ≥ 50 x) were obtained in 47/56 (83.7%) and in 8/19 (42.1%) of the samples of group #1 and #2 with DI < 5, respectively. No sample of group #3 (12 pg of template) gave a full profile. The results obtained for the other samples with higher DIs are shown in Table 1.

Genotyping results showed that full (or almost full) genotypes concordant with the expected profiles from the corresponding control samples were obtained in samples of group #1 when the amount of template ranged from 0.1 to 1.0 ng (see Table 1). For the samples of the same group with higher DIs, about 65–95% of the loci were typed and the error frequency increased to about 2–3%. As expected, the error frequency in groups #2 and #3 was even higher. Therefore, both the coverage of the loci and the frequency of drop outs are influenced both by the amount of template and by the corresponding DIs [2–5,7]. Overall, the drop out phenomena occurred mainly on loci with high molecular weight. The sister allele was totally missed in about 95% of the recorded dropouts. In addition, the coverage of the surviving allele ranged from 50 to 100 x in 36.3% of the cases (from 101-to 300 x in 49.3%; from 301 to 600 x in 11.1%; > 601 x in 3.1%). Finally, it was observed that the increase of the number of cycles up to 25–26 in the PCR resulted in a higher number of typed loci, but also introduced a higher number of artefacts (mainly allele drop-out events).

Allele drop in artefacts were scored in more than 150 cases. However, since they were clustered in a restricted selection of samples (mainly touch DNAs), it is more likely that they originated from a DNA mixture/contamination than from a PCR-MPS artefact.

4. Conclusion

Very informative genetic profiles (based on about 65–70% of the loci) can be obtained even from highly degraded samples when the amount of template ranges from 0.1 to 1.0 ng. More challenging is the analysis of lower amounts of DNA, in particular when the degradation index of the samples is high. In these cases, no more than half of the loci can be typed, while the risk of mistyping increases dramatically. It is also true, however, that the "consensus" [5,7] typing approach based on the comparison of the results from two or three replicated experiments should eliminate/minimize the risk of mistyping. When dealing with such samples, the increase of the number of PCR cycles [5,6] does not seem to improve the quality of the results, in fact, in front of the increase in efficiency of the PCR reaction (more typed loci), a higher frequency of artefacts (mainly allele drop-out events) was scored at 25–26 cycles.

Declaration of Competing Interest

None.

References

[1] S.B. Seo, J.L. King, D.H. Warshauer, et al., Single nucleotide polymorphism typing with massively parallel sequencing for human identification, Int. J. Legal Med. 127 (2013) 1079–1086.
[2] C. Børsting, S.L. Fordyce, J. Olofsson, et al., Evaluation of the Ion TorrentTM HID SNP 169-plex: a SNP typing assay developed for human identification by second generation sequencing, Forensic Sci. Int. Genet. 12 (2014) 144–154.
[3] K.B. Gettings, K.M. Kiesler, P.M. Vallone, Performance of a next generation sequencing SNP assay on degraded DNA, Forensic Sci. Int. Genet. 19 (2015) 1–9.
[4] M. Eduardoff, C. Santos, M. de la Puente, et al., Inter-laboratory evaluation of SNP-based forensic identification by massively parallel sequencing using the Ion PGM™, Forensic Sci. Int. Genet. 17 (2015) 110–121.
[5] E. Salata, A. Agostino, I. Ciuna, et al., Revealing the challenges of low template DNA analysis with the prototype Ion AmpliSeq™ identity panel v2.3 on the PGM™ sequencer, Forensic Sci. Int. Genet. 22 (2016) 25–36.
[6] Precision ID Panels with the Ion PGM™ System (Thermo Fisher), MAN0015830 Rev B.0.
[7] P. Fattorini, C. Previderé, I. Carboni, et al., Performance of the ForenSeq™ DNA Signature Prep kit on highly degraded samples, Electrophoresis 38 (2017) 1163–1174.