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Low-template methods yield limited extra information for PowerPlex® Fusion 6C profiling

Francisca Duijs, Linda van de Merwe, Titia Sijen, Corina C.G. Benschop*

Netherlands Forensic Institute, Division of Biological Traces, Laan van Ypenburg 6, 2497GB The Hague, The Netherlands

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ABSTRACT

Advances in autosomal DNA profiling systems enable analyzing increased numbers of short tandem repeat (STR) loci in one reaction. Increasing the number of STR loci increases the amount of information that may be obtained from a (crime scene) sample. In this study, we examined whether even more allelic information can be obtained by applying low-template methods. To this aim, the performance of the PowerPlex® Fusion 6C STR typing system was assessed when increasing the number of PCR cycles or enhancing the capillary electrophoresis (CE) injection settings. Results show that applying these low-template methods yields limited extra information and comes at cost of more background noise. In addition, the gain in detection of alleles was much smaller when compared to the gain when applying low-template methods to the 15-loci AmpFLSTR® NGM™ system. Consequently, the PowerPlex® Fusion 6C STR typing system was implemented using standard settings only; low-template methods were not implemented for our routine forensic casework.

1. Introduction

Forensic casework samples often contain low amounts of DNA for which standard methods for short tandem repeat (STR) typing may fail to produce a useful DNA profile. As a rule of thumb, we regard the DNA contribution of a donor to a sample low-template (LT) when the allele calls are below the established stochastic threshold. According to this view, a mixed DNA profile can have both high-template and low-template contributors. In these cases, one may consider to sensitize DNA profiling by, for example, using more amplification cycles or higher capillary electrophoresis (CE) injection settings [1–12]. We refer to this as applying a low-template method.

From 2010 to 2017 the Netherlands Forensic Institute applied the five-dye AmpFLSTR® NGM™ short tandem repeat (STR) typing system in forensic casework. For the majority of samples standard settings are applied, but when applicable, increased cycling or enhanced CE is conducted [13,14]. In 2017, the NGM system was replaced by the six-dye PowerPlex® Fusion 6C multiplex system, which includes a total of 27 instead of 16 loci (with 23 instead of 15 autosomal STR loci). This increased number of loci increases the amount of information that may be obtained from case samples [15]. In this study, we assessed whether the amount of allelic information can be further increased by applying LT methods, which was part of our in-house validation study. To this aim, the number of PCR cycles was increased from 29 to 32, 33 or 34 and the CE settings were increased from 1.2kV24s to 3.6kV24s. Results are used to infer whether implementation of one or more LT methods (besides standard profiling settings) for PowerPlex® Fusion 6C profiling is regarded useful in routine forensic casework.

2. Materials and methods

Four sets of DNA samples, denoted set A, set B, set C and set D, were created according to Table 1 (further details regarding set D can be found in supplementary Table 1). Pristine DNA samples (Promega 2800 M and Thermo Fisher DNA007 control DNAs) as well as mock casework samples were used and genotypes of all donors were known (see supplementary Table 2 for the genotype profiles of 2800 M and DNA007). The mock casework DNA samples were obtained from a bone from an excavated human grave [16]. Varying amounts of bone powder extracted with slightly different extraction methods were used, resulting in 12 DNA extracts. Quantification of the DNA extracts was performed using an ALU assay that has a lower detection limit of 0.5 pg/μL human DNA [17,18]. DNA extracts were grouped into categories according to their quality and measured quantity; *i.e.* category 1) DNA concentration of 2–10 pg/μL, category 2) DNA concentration below 2 pg/μL and category 3) strongly inhibited DNA samples (known from earlier experiments; precise nature of inhibition not known). A reference profile of the bone was obtained from previous DNA extracts from organs of the same individual [16].

Replicate PowerPlex® Fusion 6C (Promega, PPF6C) and AmpFLSTR®

* Corresponding author.
E-mail addresses: f.duijs@nfi.minvenj.nl (F. Duijs), l.v.d.merwe@nfi.minvenj.nl (L. van de Merwe), t.sijen@nfi.minvenj.nl (T. Sijen), c.benschop@nfi.minvenj.nl (C.C.G. Benschop).

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Table 1
Overview of samples and PCR and CE settings used in this study.

Sample set	Sample type	Amount of DNA (pg) in the PCR		Number of DNA samples	Number of replicates	Number of profiles (Total 438)								
						PPF6C					NGM			
						Cycles	29	29	32[a]	33[b]	34	29	29	29	34[c]
						kV	1.2	3.6	1.2	1.2	1.2	3	3	9	3
		2800M	DNA007			Sec.	24	24	24	24	24	5	15	10	5
A	1 donor (2800 M)	6	–	4	3		12	12	12	12	12	–	–	–	–
		12	–												
		24	–												
		48	–												
B	1 donor (2800 M)	6	–	3	15		–	–	45[d]	45[d]	–	–	–	–	–
		12	–												
		18	–												
C	2 donors (2800 M + DNA007)	24	6	3	4		12	12	12[d]	–	–	–	–	–	–
		48	12												
		60	12												
D[e]	Mock casework: 1donor: 2–10 pg/μL	[f]		4	3		12	12	12	–	–	12	12	12	12
	Mock casework: 1donor: < 2pg/μL	[f]		4	3		12	12	12	–	–	12	12	12	12
	Mock casework: 1donor: Inhibited	[f]		4	3		12	12	12	–	–	12	12	12	12

[a] Sample sets A&B 32 cycles, sample sets C&D: 29 cycles followed by three additional cycles.
[b] Sample set A: 33 cycles, sample set B: 32 cycles followed by one additional cycle.
[c] 29 cycles followed by five additional cycles.
[d] At the −1 and +1 repeat unit stutter position the applied stutter filters were 1.5-fold higher when compared to manufacturer's recommended stutter ratios.
[e] Analysis of mock casework samples used in-house determined locus specific stutter filters.
[f] Further detailed information regarding mock casework samples can be found in Supplementary Table 1.

NGM™ (Thermo Fisher, NGM) PCR amplifications were performed using settings as presented in Table 1. PPF6C and NGM PCR followed the manufacturer's recommendations, except for a reduced total PCR volume of 12.5μL for PPF6C and, for some samples, a higher number of PCR cycles (Table 1). A higher number of PCR cycles was performed either uninterrupted (indicated as 29, 32, 33 or 34 cycles) or as a 29+ or 32+ approach which means that after 29 or 32 cycles 1μL of PCR product is removed for CE analysis after which the remaining PCR product (11.5μL) is subjected to one, three or five additional PCR cycles which is indicated as 29 + 3, 29 + 5 or 32 + 1 cycles. The advantage of this approach is that the enhanced CE and increased cycling profiles have the exact same basis as the standard 29c profiles, which allows a straightforward comparison. In total, 294 PPF6C en 144 NGM profiles were generated.

CE analysis was performed on an ABI3500xL (Thermo Fisher) for PPF6C PCR products using per run 9.6μL (1.2kV24s) or 9.8μL (3.6kV24s) HiDi formamide, 0.4μL (1.2kV24s) or 0.2μL (3.6kV24s) WEN ILS 500 and 1μL of PCR product or allelic ladder. NGM PCR products were run on a ABI3130xL apparatus (Thermo Fisher) as described in [13].

PPF6C profile analysis was performed using GeneMarker HID v2.9.0 (settings: Auto Range Raw data analysis; Smooth + Superior smoothening; Cubic Spline; Auto Range Allele Call; 2% heterozygote imbalance) using dye specific detection thresholds (FL = 45, JOE = 50, TMR = 45, CXR = 80, TOM = 40 relative fluorescent units (RFU)) and locus specific stutter ratios as recommended by the manufacturer, unless indicated otherwise (see Table 1). Ref. [13] describes the thresholds and settings which were applied for NGM profile analysis using GeneMapper ID-X.

3. Results and discussion

Standard settings for PPF6C PCR and CE and four LT-methods were applied to the samples described in Table 1. Comparison between the effectiveness of LT-methods involved the determination of the percentage of detected donor alleles (homozygous alleles counted as two alleles); average peak height (note that PPF6C amplified products are run on a 3500xL instrument and that peak heights range from the dye-specific detection threshold to ~ 30,000 RFU); the number of noise peaks (which mainly occur when samples with limited amplification levels are analysed leading to calls not representing alleles such as baseline pull-ups, spikes, bleed through peaks, aberrantly shaped peaks and -1 base calls ahead of allele calls that probably reflect non-adenylated products, see supplementary Fig. 1B), non-filtered stutter peaks and drop-in alleles (which are alleles not corresponding to the donor such as alleles from the environment or contamination). With increasing amounts of pristine DNA (set A, Table 1), more alleles are detected (Fig. 1A), and the percentage of detected alleles increases slightly when a LT method is applied (Fig. 1A). Especially when the highest cycle number (34) is applied very high numbers of noise peaks and elevated stutters are obtained, even with low DNA inputs, (Fig. 1A) that hamper profile analysis. Consequently, 34c was excluded from further analyses.

As a next step, the performance of 32c or 33c was examined in more detail by profiling sample set B (Table 1). Percentages of detected alleles were similar for both methods. Peak heights were, as expected, approximately two-fold higher for 33c than for 32c (Fig. 1B), but also the stochastic threshold was found to be higher when using 33 cycles (Fig. 1A). The stochastic thresholds were defined during the in-house validation of the PPF6C kit and set at a level below which 99% of single alleles of heterozygous loci occurred [13]. When alleles have heights below the stochastic threshold, stochastic effects such as drop-out and peak imbalance [19] are likely and should be accounted for during profile interpretation. Even though 33c yielded higher peak heights than 32c, for both methods most of the alleles remained below their specific stochastic threshold (97.5% and 97.3% for 33c and 32c, respectively, using the highest DNA input of 18 pg). Furthermore, 33c yielded more background noise when compared to 32c (Fig. 1B, for an example of noise patterns see Supplementary Fig. 1). These results together provided a preference for 32c over 33c, and 33c was excluded from subsequent analyses.

The mixed samples in sample set C (Table 1) were amplified in