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Analysis and interpretation of mixed profiles generated by 34 cycle SGM Plus® amplification

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ABSTRACT

Interpretation rules for standard 28 cycle PCR have been described previously for the analysis of mixed STR profiles. In this study the same guidelines are applied to 200 mixtures derived from pairs of known donors combined in ratios of 1:1, 2:1 and 5:1 which have been profiled in duplicate with SGM Plus® at total inputs ranging from 1 ng to 50 pg. The paired profiles were distributed among 35 FSS (Forensic Science Service) reporting officers trained in low copy number (LCN) interpretation who analysed them blind following standard casework procedures. Based upon the results from initial duplicate 34 cycle PCR reactions, the reporting officers made appropriate decisions regarding the benefits of processing the reserved third aliquot. Using the combined results, 49 consensus profiles were successfully resolved into major and minor contributor peaks. This demonstrates the reliability of the interpretation rules used in standard 28 cycle SGM Plus analysis when applied to 34 cycle generated profiles by trained and experienced reporting officers. No minor contributor peaks were assigned to a major profile in the final reported results. Those profiles which did not show sufficiently marked and consistent differentiation into major and minor peaks would have been correctly resolved if the profile of one contributor (e.g. the "victim") was known.

1. Introduction

Casework stains may contain limited amounts of DNA suitable for analysis if they are very small or degraded. Several strategies for the analysis of such low template DNA (LT-DNA) samples have been developed by enhancing the sensitivity of standard STR analysis protocols, these include increasing the amount of PCR product analysed by prolonging injection times or concentrating the substrate DNA into a single PCR reaction [1–4]. A concern with such approaches is that contamination during the analytical stages might go unrecognised and contribute to the single profile analysed. The low copy number (LCN) protocol adopted by The Forensic Science Service® (FSS) in 1999 maximizes sensitivity by increasing SGM Plus cycle number from 28 to 34 potentially enabling the detection of single template molecules whilst mitigating the consequences of contamination during analysis by performing the post-extraction stages in duplicate [5]. Only those alleles observed in both duplicate PCRs are included in the reported consensus profile, thereby excluding those resulting from non-donor "drop-in" and sporadically contaminated consumables [6]. Subsequent interpretation of profiles derived from 34 cycle PCR uses the same well-established rules based on quantitative aspects of the DNA profile that are used with the standard 28 cycle protocol with the additional requirement that the quantitative aspects should be reproducible between duplicate amplifications. The relative values of peak height and area are used to identify individual major and minor component profiles building upon the correlation between input amounts and peak height that is still maintained at 34 cycles (R. Puch-Solis et al., personal communication).

Regardless of the method of production, e.g. 28 or 34 cycles, some DNA profile mixtures can be easily resolved if one of the contributors can be logically inferred, for example the victim from a vaginal swab. In such situations it is common practice to subtract the known contributor's profile in order to identify that of the second, taking into consideration that alleles may be shared by both. Without prior knowledge resolving mixtures is harder, particularly those which are evenly balanced due to similar input quantities or with more than two contributors. However, where the contributions of the donors differ markedly in amount, the profile can often be resolved on the basis of signal strength into major and minor (strong and weak) contributions.

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In order to resolve mixtures confidently the quantitative aspects must be reproducible, a characteristic which decreases as the available template is reduced since stochastic variation concurrently increases. At high template inputs (greater than 400 pg) the reproducibility of single source samples is a statistical consequence of adding more than 60 copies of each allele to the PCR reaction which is sufficient to ensure that variation in the signal strength between alleles is limited [7]. When this amount of DNA is available SGM Plus amplification would usually be carried out at 28 cycles with no need for replication as a similarly balanced result would be expected if the test were repeated. With lower single source inputs (below 200 pg and less than 30 copies of each allele) stochastic processes are expected to produce some variation in relative peak heights at heterozygous loci between replicate PCRs, which as the input declines further may eventually result in allelic drop-out when the sampling process fails to include a template copy of an allele from the contributor's profile. At such low levels the risk that accidental contamination of the sample could introduce a non-donor allele at the level of the sampled profile becomes significant; consequently when handling low copy number samples, in addition to working in a highly clean environment, duplication of the profiling process is used to minimise the risk of falsely including a rare contaminant (drop-in) allele by only incorporating the alleles in a consensus profile that were observed in more than one PCR.

It follows that, in theory, if a high template and low template profile are combined then the major contributor's profile's morphology and quantitative characteristics will remain constant in successive tests whereas the minor contributor's profile will vary. This consideration applies to mixtures containing high and low templates regardless of whether the profile is produced by a standard 28 cycle test or a more sensitive 34 cycle LCN test [8]. Therefore when the LCN protocol was established it was not considered necessary to conduct any further validation on mixture interpretation since the duplication of any major component profile would demonstrate that the major component of the mixture was above the stochastic threshold and in essence was not low template (and thus standard interpretation rules applied). The interpretation of the minor component on the other hand would be expected to be affected by stochastic effects and might require further interpretation considerations. The recent introduction of Quantifiler® Duo as a quantification technique can further assist in establishing whether components of a mixture are approaching the stochastic threshold and require additional interpretational consideration.

In order to provide data to further demonstrate this principle we have produced various mixtures of known individual profiles in different ratios and at different dilutions at and around the low template levels at which large stochastic effects and allelic drop-out can be observed (the stochastic threshold). These samples have been processed and analysed within the casework units in accordance with standard FSS procedures. The data have been interpreted 'blind', i.e. in isolation of the reference profiles used to construct the mixtures, by DNA reporting officers (ROs) from across the FSS who have been trained and are deemed competent in the interpretational issues encountered when profiling LT-DNA.

In forensic casework, ROs may be required to interpret DNA profiles from casework stains for

(a) evidential use, that is, for comparison with named DNA reference samples and possible statistical evaluation,
(b) intelligence use, that is, for possible comparison with a DNA Database whether by permanent loading or by a one-off speculative search.

Their role includes an assessment in each instance of whether the result can be reliably interpreted for the required purpose. Since no case information and no reference samples were provided in this study, the ROs assessed how the result could be interpreted by considering whether a major profile suitable for NDNAD searching could be elicited, whether there was no major profile but the result would be suitable for evaluation if references were available, whether a weaker profile suitable only for more limited intelligence purposes could be elicited, or whether the profile was unsuitable for further interpretation. The RO's interpretation was then returned to the trial coordinators for comparison against the known profiles of the donors.

This work extends that performed for standard 28 cycle SGM Plus mixture analysis [9,10] and provides a further demonstration of the validity of mixed profile assessment [11].

2. Materials and methods

2.1. Samples

Initially buccal scrapes from four donors were paired to create two sets of mixtures for testing at a wide range of mixture ratios (20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10 and 1:20) and different total concentrations (1 ng, 500 pg, 250 pg, 100 pg, 75 pg, 50 pg and 25 pg). This demonstrated that the range from 1 ng to 50 pg at mixture ratios between 1:1 and 5:1 regularly produced interpretable LCN mixtures to which both donors had contributed and was also representative of the mixtures generally obtained in casework. For the second phase, which is reported here, 16 donors were assigned randomly to eight pairs. Each donor provided several buccal scrapes (Whatman® OmniSwab™) which were extracted using the Qiagen® BioRobot EZ1 and Investigator bead chemistry kit. The pooled extracts provided a large volume of DNA from each donor of sufficient concentration to set-up all the dilutions. To ensure accuracy the extracts were quantified in quadruplicate using the Applied Biosystems® Quantifiler® Duo Kit and ABI 7500 Real-Time PCR System.

All the samples were normalised to 1 ng/μl and re-quantified before each of the mixture ratios were set-up as a 1.0 ng/μl bulk stock. These bulk volumes were then diluted to a concentration such that the addition of 1.0 μl to the amplification reaction would give each of the target template levels. The chosen total inputs were 1 ng, 500 pg, 250 pg, 100 pg and 50 pg at mixture ratios of 5:1, 2:1, 1:1, 1:2 and 1:5, i.e. a 5:1 mixture containing 1 ng total DNA would consist of 833 pg of major component and 167 pg of minor component (see Table 1).

2.2. Sample processing

All mixture samples were tested in duplicate using the Applied Biosystems AmpFℓSTR® SGM Plus PCR Amplification Kit at a validated volume of 25 μl. Amplification reactions were run using the manufacturer's cycling parameters for 34 cycles of PCR on MJ Research Tetrad thermal cyclers. Capillary electrophoresis was carried out on ABI Prism® 3130xl Genetic Analyzers (Applied Biosystems) using 1.5 kV injection voltage for 10 s.

Table 1
The relative inputs of the major and minor donor in mixed profiles created with different inputs and mixture ratios.

Mixture ratio (A:B)	DNA input from contributor A (pg)				
	1000	500	250	100	50
5:1	833	417	208	83	42
2:1	667	333	167	67	33
1:1	500	250	125	50	25
1:2	333	167	83	33	17
1:5	167	83	42	17	8