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The effect of whole genome amplification on samples originating from more than one donor

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Abstract. In this study, the GenomiPhi™ DNA Amplification Kit (Amersham Biosciences) was used to investigate the potential of whole genome amplification (WGA) when considering samples originating from more than one donor. DNA was extracted from blood samples, quantified and normalised before being mixed in ratios of 1:1, 1:3, 1:7 and 1:15. Proportions were checked using standard STR analysis before being subjected to WGA. Once amplified using GenomiPhi™, the ratios were again checked. Relative proportions were found to be maintained in the 1:1 and 1:3 ratios following WGA; the observed peak ratios were found to match the expected peak ratios regardless of the starting concentration of DNA. With samples mixed in the ratio of 1:7 and 1:15, and when the concentration of starting material was at the manufacturer's lower limit, too few minor component peaks were found to allow for statistical analysis. With an initial template exceeding 1 ng/μL there was an increase in problems associated with profile interpretation but the results obtained indicated that mixture proportions could be quantifiably maintained. © 2006 Published by Elsevier B.V.

Keywords: Whole genome amplification; GenomiPhi™ DNA Amplification Kit; Mixture analysis

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1. Introduction

Limited starting material is a common problem in forensic science. Samples are often compromised in terms of quality or quantity (sometimes both) and the possibility of contribution by more than one donor is a necessary and frequent consideration. Whole genome amplification (WGA) offers the opportunity to create a 'stock' of starting substrate on which to perform subsequent testing and provides an interesting avenue of investigation for the forensic scientist. Its potential to deal with mixed samples is of particular interest and the research presented here looks at the ability of WGA (using the GenomiPhi™ DNA Amplification Kit, Amersham Biosciences) to cope with samples originating from more than one individual. The GenomiPhi™ DNA Amplification Kit uses bacteriophage Phi29 DNA polymerase enzyme to amplify the sample DNA by strand displacement amplification. The starting source of DNA is not restricted to purified genomic DNA. Whole blood, blood on paper, buccal swabs and frozen animal tissue are supported by the manufacturer with a sample input of 1 ng of human genomic DNA reported as having provided successful results, giving an expected yield of 4–7 μg.

2. Materials and methods

2.1. Mixture preparation

Blood samples were taken from four individuals (A, B, C and D) and DNA extracted using the QIAamp® DNA Mini Kit (Qiagen). The extracts were quantified (in duplicate) using the Quantifiler™ Human DNA Quantification Kit (Applied Biosystems) on the ABI PRISM® 7700. Following quantification, the extracts were normalised and extract A was mixed with B whilst extract C was mixed with D. In each case the samples were combined in the ratios 1:1, 1:3, 1:7 and 1:15. The mixture proportions were verified by performing routine amplifications on 1 ng/μL aliquots using both the AmpF/STR® SGM Plus® PCR Amplification Kit (Applied Biosystems) and the PowerPlex® 16 System (Promega). Peak areas were used to calculate the observed ratios (shown in Table 1).

2.2. Whole genome amplification

Aliquots of the prepared mixtures were distributed to participating laboratories and the following method used by all, in conjunction with the individual laboratory's standard

Table 1
Observed ratios in artificial mixtures: a summary of the statistical analysis

Proportion contributed by minor component				
Ratio	Expected	Observed		
		Pre-GenomiPhi™	Post-GenomiPhi™	
			1 ng/μL	14 ng/μL
1:1	0.5000	0.49 (0.44–0.54)	0.49	0.51
1:3	0.2500	0.27 (0.23–0.30)	0.20	0.25
1:7	0.1250	0.15 (0.12–0.18)	ND	0.14
1:15	0.0625	0.12 (0.10–0.14)	0.32	0.10

ND = not determined.

operating procedures. Each aliquot was divided into two with one being used for WGA. WGA was performed by adding the minimum concentration of starting material recommended by the manufacturer (1 ng/μL). The reaction was also performed by adding DNA at concentrations known to exceed this minimum value. The remainder of the protocol was performed according to manufacturer's guidelines. Clean-up was not performed and samples were quantified in duplicate using the laboratories' routine methodology. The majority of samples gave a yield of 100–150 ng/μL.

2.3. Amplification

The remainder of the original artificial mixture aliquot and the WGA extracts was amplified using the individual laboratory's standard amplification kit and conditions.

3. Results and discussion

Table 1 shows a summary of the statistical analysis of expected and observed peak areas. Results obtained using initial templates of 1 ng/μL and of 14 ng/μL are illustrated in the table. Relative proportions were found to be maintained in the 1:1 and 1:3 ratios following WGA; the observed peak ratios were found to match the expected peak ratios regardless of the starting concentration of DNA. With samples mixed in the ratio of 1:7 and 1:15, and when the concentration of starting material was at the lower limit, too few minor component peaks were found to allow for statistical analysis. With an initial template exceeding 1 ng/μL, there was an increase in problems associated with profile interpretation but the results obtained indicated that mixture proportions could be quantifiably maintained.

3.1. Interpretation problems

WGA led to significant problems in interpreting the electropherograms. Split peaks were not uncommon (despite standardisation of concentration). The stutter proportion was greatly increased. Additionally extra peaks were observed. Although a starting template exceeding 1 ng improved the number of alleles observed within the mixture, in the WGA samples this also gave rise to peaks with atypical architecture. The result of all these artefacts meant that analysis of unknown mixtures became problematic.

4. Conclusions

For ratios of 1:1 and 1:3 it was found that initial proportions could be maintained following WGA. This study also showed that mixture proportions could be quantifiably maintained with an initial template exceeding 1 ng/μL. It was found that the concentration of starting material was important in cases where the minor component contributed 12.5% or less of the total mixture. In these cases, a 1 ng/μL template seemed to result in preferential amplification with the loss of alleles from the minor contributor. Inconsistent profiles were also produced and peak imbalances made interpretation difficult.

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