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Whole genome amplification—the solution for a common problem in forensic casework?

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Abstract. To assess the quality of amplified DNA obtained by whole genome amplification, 17 independent STR loci have been typed using two multiplex kits. Results have been compared for correct genotypes, heterozygous peak balance and allelic dropout. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

A typical problem in forensic stain cases as well as in mass disaster victim identification is the lack of sufficient amounts of genomic DNA in adequate quality. Furthermore, the success of the PCR-based typing methods has led to a dramatic increase in casework samples where only a few nanograms of DNA are available for typing. A possible solution for this ''bottleneck'' of forensic DNA analysis could be the amplification of the entire genomic DNA prior to locus-specific PCR analysis. Recently, this approach has been improved considerably by using the highly processive Phi29 DNA polymerase which can be used in an isothermal ''rolling circle amplification'' [1]. Briefly, amplification of genomic DNA is initiated by introducing random hexamer primers. The Phi29 polymerase uses a free 3′ end as starting point, but the replication does not stop when a 5′ end of double-stranded DNA is encountered. Phi29 will displace this strand from the 5′ end and continue with the replication process. New hexamers will bind to the displaced strand and initiate further replication sites leading to an almost exponential amplification reaction. Furthermore, Phi29 polymerase has an approximately 10-fold increased replication fidelity compared to Taq polymerase. Therefore, we have

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Table 1
Whole genome amplification yields from P118 cell line DNA

DNA input	5 ng	500 pg	50 pg	5 pg
Repli-g 625S	511 ng	530 ng	471 ng	464 ng
GenomiPhi	224 ng	204 ng	184 ng	180 ng

tested whole genome amplification regarding its suitability for multiplex STR typing systems.

2. Material and methods

The two following kits were tested: Repli-g 625S (Molecular Staging) and GenomiPhi (Amersham Biosciences). The two human cell lines HepG2 and P118 were used in dilutions down to 5 pg. The amplification conditions were according to the manufacturers' instructions (16 h at 30 °C). The concentrations of the amplified samples were determined by measuring several independent dilutions by fluorometry. The amplified DNA was subjected to multiplex STR typing using both the PowerPlex 16 (Promega) and the AmpFlSTR SGM Plus kits (Applied Biosystems). Each STR analysis was carried out twice for each cell line and dilution, as well as using both kits. Results were assessed by GeneScan computer analysis and by comparing the relative peak heights of the two alleles of selected heterozygous STR loci.

3. Results and discussion

Whole genome amplification was found to be very efficient. The resulting yield of amplified DNA appeared not to be clearly correlated with the amount of DNA present in the reaction, although the overall yield was about 2-fold higher for Repli-g in comparison to GenomiPhi (Table 1). This may indicate that an amplification plateau was reached in all dilutions tested, even when only 5 pg—the equivalent of a single genome—were used. The presence of high molecular weight DNA was clearly demonstrated in all reactions after agarose gel electrophoresis. However, genetic typing of the amplified DNA is required to assess the specificity and the quality of the reaction products.

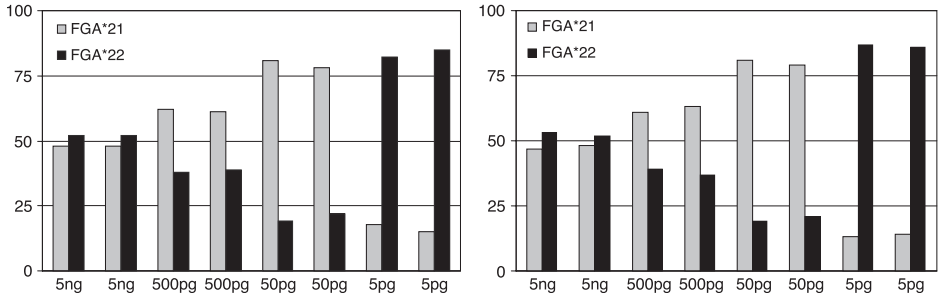

Fig. 1. Repli-g 625S: P118 DNA and FGA peak balance (%) using 5 ng to 5 pg for amplification, left panel: SGM Plus kit; right panel: PowerPlex 16 kit.

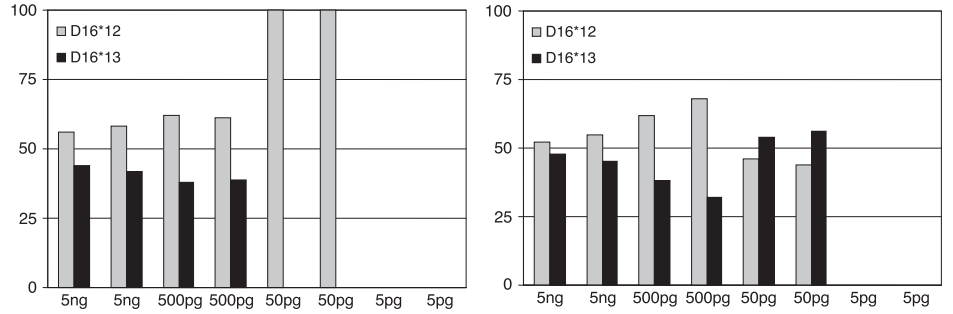

Fig. 2. P118 DNA and SGM Plus D16S539 peak balance (%) using 5 ng to 5 pg for the Repli-g kit (left panel) and the GenomiPhi kit (right panel).

After completion of multiplex STR typing, results from several loci with heterozygous genotypes were further studied to assess peak balance, allelic dropouts and other artefacts. It was found that reliable typing results could be obtained from amplified DNA generated from 500 pg genomic DNA. Dropouts started to occur at 50 pg, and more dramatically at 5 pg. "Drop in" alleles were not observed. In Fig. 1, the results for the two STR multiplex kits for the FGA locus are shown for all DNA concentrations tested in duplicate. It becomes evident that the peak balance is distorted as well as reversed regarding the stronger peak for the 50 and 5 pg reactions, respectively. Also, there is no difference between the two STR kits once the genomic DNA has been amplified. Fig. 2 depicts the occurrence of allelic dropout for the D16S539 locus at 50 pg for the Repli-g kit only, and complete locus dropout at 5 pg for both kits. Obviously, this loss of information occurs due to stochastic effects during genome amplification. Once the amplified DNA has been generated, the stochastic effects lead to genomic regions which may be over- or underrepresented.

It will be important to generate more data on reproducibility to better understand these effects. Whole genome amplification could become a tool for saving precious DNA samples for research purposes, and maybe—once the reliability has been demonstrated—even for limited crime case samples. If stain DNA is sufficient for only two to three direct PCR typings, genome amplification may be used to produce a batch of DNA for further studies without the need to decide about the loci to be analyzed.

Reference

[1] F.B. Dean, et al., Rapid amplification of plasmid and phage DNA using Phi 29 DNA polymerase and multiply-primed rolling circle amplification, Genome Res. 11 (2001) 1095–1099.