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Application of whole genome amplification for forensic analysis

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Abstract. Fundamental to most forensic analyses is the availability of genomic DNA of adequate quality and quantity. To perform a multitude of genetic analyses and assays requires a sufficiently large amount of template. However, DNA yield from forensic samples is frequently limiting the extent of genetic typing. A possible solution to overcome this "bottleneck" of forensic and paleoarcheological DNA analyses could be the amplification of the entire genomic DNA prior to locus specific PCR analysis. Whole Genome Amplification appears to be a promising tool to obtain sufficient DNA amounts from forensic samples of limited quantity.

Keywords: Whole genome amplification; WGA; MDA; Forensic DNA; STR typing

1. Introduction

The WGA method is based upon the "Strand-Displacement Amplification" approach used in rolling circle amplification [1]. Previously, we had performed an initial whole genome amplification experiment regarding its suitability for DNA diluted to 5 ng, 500 pg, 50 pg and 5 pg, and for multiplex STR typing systems [2]. Preliminary results had

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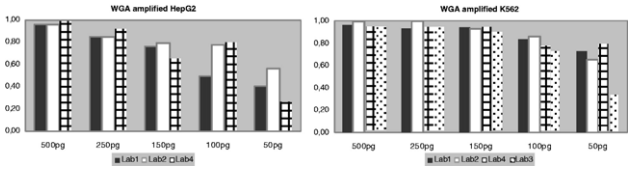

Fig. 1. Proportions of STR profiles obtained overall for WGA amplified HepG2 and WGA amplified K562.

indicated WGA to be very efficient. However, the limit of the DNA quantity suitable for whole genome amplification seemed to be between 500 pg and 50pg diluted DNA. Therefore the main purpose of the present study was to investigate its sensitivity, accuracy and suitability for DNA diluted with quantities of 50, 100, 150, 250 and 500 pg. Therefore, we have generated more data by performing a systematic sensitivity study carried out in parallel in four laboratories.

2. Material and methods

We have carried out the sensitivity study using two cell lines, HepG2 and K562 DNA diluted to 50–500 pg, respectively. The whole genome amplifications were performed in Lab 1 and Lab 2 five times independently for both cell lines and all dilutions using the GenomiPhi Amplification Kit (Amersham Biosciences); the amplification conditions were according to the manufacturer's instructions. The amplified DNA was diluted and subjected to multiplex STR typing using the AmpFlSTR SGM Plus kit (ABI). The PCR was carried out twice for each WGA DNA.

Results were compared for correct genotypes, heterozygous peak balance and allelic dropout. As further control, the results from the two laboratories were assessed by comparing also with reference results processed in Lab 3 and Lab 4.

3. Results and discussion

WGA was very efficient with an average yield of 225–350 ng/μl. WGA yield was not correlated with the amount of input DNA. The proportion of the successfully typed STR profiles decreased with reducing the input DNA (Fig. 1).

The results of 500 pg DNA subjected to WGA from both laboratories resulted in a very high genotyping efficiency with rates of 96–99% for both cell lines. STR typing using 250 pg of input DNA provided reliable results. From WGA amplified K562 DNA diluted to 250 and 150 pg high STR call rates of 93–100% could be obtained, while for the same dilutions of the HepG2 DNA only 76–85% were observed. Stochastic effects increased

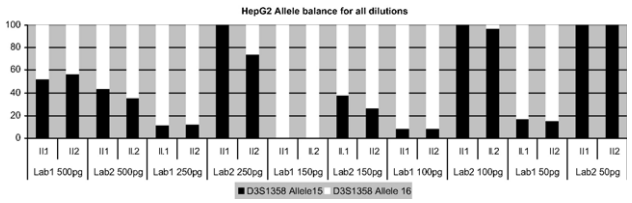

Fig. 2. Example for allelic imbalance for locus D3 of WGA amplified HepG2 DNA, obtained by both laboratories for one WGA (No. II) and duplicate PCR (No. 1 and 2) and all dilutions.

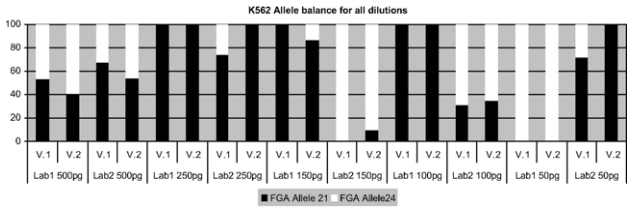

Fig. 3. Examples for allelic imbalance for locus FGA of amplified K562 DNA, obtained by both laboratories for one WGA (No. V) and duplicate PCR (No. 1 and 2) and all dilutions.

with decreasing the input DNA. Allelic imbalance increased with reducing the amount of the input DNA, strong allelic imbalances appeared at 250 pg (Figs. 2 and 3).

An increasing number of allele dropouts became evident as the amount of DNA in the WGA reaction was reduced to 250 pg, and occurred more strongly at 100 pg (14–35% allele dropout for K562 and 22–60% using HepG2). The percentage called in Lab3 using WGA amplified K562 DNA dilutions and in Lab4 using both DNA confirmed the tendency obtained by the other two laboratories (Fig. 1).

Several authors have suggested the advantages of MDA as a method of choice to amplify DNA suitable for analysing SNPs and the compatibility with high-throughput genotyping techniques. However, the accuracy of the method has been assessed only for using 20 ng DNA from fresh blood samples, fresh tissues and material for human disease research and preimplantation genetic diagnosis [3].

In the present study, the applicability of MDA-WGA using genomic DNA from human cell lines in controlled dilutions from 500 pg down to 50 pg has been investigated, which is consistent with typical forensic material, where a lack of sufficient amounts of DNA in adequate quality is typical. In general ~300 ng/μl were obtained, independent of the amount of target DNA. However, reliable STR amplification was dependent on the DNA quantity used for WGA. Consistent and reliable STR typing was only obtained using 500 pg genomic DNA. Dropouts and allelic imbalance started to occur at 250 pg and more dramatically at 100 and 50 pg. Therefore, the usefulness of WGA in forensic casework is limited. However, the method may be very useful for saving rare samples and for “immortalization” of clinical samples [4] provided that the DNA is of adequate quality. These results are completely congruent with those obtained by others [5,6] and show that sensitivity and specificity of the WGA protocols depend to a large extent on the amount of the starting g-DNA.

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