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Whole-genome amplification: relative efficiencies of the current methods

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Abstract

Whole genome amplification (WGA) has emerged as a fundamental method for DNA analysis from limited quantities of genomic DNA in forensic analysis and disease gene discovery. Several strategies for WGA have been developed during the past decade, each with variable fidelity, yield and coverage of the amplified genome. In the search for a reliable and robust WGA method for genotyping short tandem repeat (STR) loci and single nucleotide polymorphic (SNP) markers, we initially tested four common methods, viz., degenerate oligonucleotide primed PCR (DOP), primer extension preamplification (PEP), improved-PEP (I-PEP) and multiple displacement amplification (MDA), typing the 13 CODIS tetranucleotide repeat loci. The results showed among all methods, I-PEP and MDA have higher genomic coverage. DOP and PEP produced locus and allelic dropouts. Therefore, we emphasized on the evaluation of I-PEP and MDA protocols, which shows that the two methods have their relative strengths and weaknesses. In general, the product yield of MDA is higher than that of I-PEP. However, the specificity of the I-PEP products appears to be higher, particularly in the analysis of STR loci. In the analysis of SNP markers, some loci amplify better using products obtained from I-PEP whereas some worked better with MDA. Our analyses also demonstrate that blood spots on FTA cards are a more efficient source of DNA for I-PEP as compared to MDA, especially for STR analysis.

Keywords: Whole genome amplification (WGA); Improved primer extension pre-amplification (I-PEP); Multiple displacement amplification (MDA); FTA card; Genomic DNA (g-DNA); Forensic DNA analysis

1. Introduction

In forensic DNA analysis, a typical requirement is the availability of about 60 or more copies of good quality genomic DNA (g-DNA). However, in reality, this requirement is more often not met with the recovered evidence material consisting of miniscule amounts DNA posing a challenge in the effective application of standard laboratory protocols in marker DNA analysis. In a separate context, with the emerging prospect of whole-genome association studies for disease gene discovery, using hundreds of thousands of genetic markers becoming a reality and the availability of reagents and high-throughput genotyping technologies for this purpose, a likely impeding factor holding back on this potential would be the limited quantity of source g-DNA available for analysis. Whole-genome amplification (WGA) has, therefore, emerged as a critically fundamental method for reproducing abundant quantities of DNA from its source with enormous potential in forensic analysis and gene discovery. In fact, effective implementation of a robust WGA method would have tremendous impact on all fields involving genetic marker analysis, that include clinical genetics, molecular epidemiology, population and evolutionary genetics. During the past decade, several WGA methods have been developed, e.g. degenerate oligonucleotide primed PCR (DOP) [1,2] and its modifications, viz., long-DOP-PCR [3] and long products from low DNA quantities DOP-PCR (LL-DOP-PCR) [4]; primer extension preamplification (PEP) [5] and improved-PEP (I-PEP) [6]. All of these methods are PCR-based. Recently, a non-PCR based method was developed in a simple isothermal reaction called multiple displacement amplification (MDA) [7,8].

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Although the potential of the various WGA methods has been tested [9–13], each has its inherent strengths and limitations. In this study, we have evaluated the relative efficiencies of the available WGA methods with respect to their product yield, genome coverage, sensitivity and fidelity. This evaluation is primarily based on the analysis of a set of microsatellite markers using pre-amplified WGA products obtained by various protocols. These microsatellites are the 13 short tandem repeat (STR) loci that form the core of the Combined DNA Index System (CODIS). With a robust protocol optimized for relatively equal amplification of the alleles at all markers in addition to the gender-determining amelogenin locus, developed primarily for use in forensic analysis [14], the CODIS markers provide an effective system for assessment of the WGA methods. Our preliminary results showed variable allelic dropouts using amplified PEP and DOP products (data not shown). However, amplification of all of the 13 STR loci and the amelogenin marker was achieved using I-PEP and MDA DNA. Therefore, for further analysis, we chose to limit our efforts to the evaluation of the WGA products obtained by using I-PEP and MDA protocols. We provide empirical data on product yield, sensitivity and differential amplification of alleles in heterozygotes resulting in allelic imbalance and comparative evaluation of other parameters in the analysis of the STR loci using these methods. We further demonstrate that blood samples spotted on FTA cards can be effectively subjected to both I-PEP and MDA.

Additionally, we have typed 30 single nucleotide polymorphisms (SNPs) spanning the entire genome using g-DNA samples and their corresponding I-PEP and MDA products. This is to demonstrate the effectiveness of the two WGA protocols in the analysis of SNP markers.

2. Material and methods

2.1. Genomic DNA (g-DNA)

We used g-DNA from four different sources: (i) CEPH DNA samples obtained commercially; (ii) DNA extracted from whole blood using the kit from Gentra Systems; (iii) DNA extracted from blood spots on Whatman FTA cards using the manufacturer's protocols; and (iv) 45 randomly chosen DNA samples from our laboratory for typing the SNP markers. Negative controls used in our study are described in the legend of Fig. 1.

2.2. Whole genome amplification protocols

2.2.1. Improved-primer extension preamplification (I-PEP)

We have followed the I-PEP protocol as described in Dietmaier et al. [6], which is essentially a modification of the original PEP amplification method developed by Zhang et al. [5]. PCR was conducted in a 30 μl reaction containing 0.01–50 ng of genomic DNA, 500 pmol of 15-base totally degenerate random primer, 200 μM each dNTPs, 2.5 mM $MgCl_2$, 1 μl of 10×PCR buffer, 1.5 μl gelatin (1 mg/ml), and 2.1 units of high fidelity *Taq* DNA polymerase (Expand High Fidelity PCR System, Roche Diagnostics). Samples were amplified in a 9600 Perkin-Elmer PCR thermal cycler with the following cycling conditions: after a pre-denaturation step of 2 min at 94 °C, 50 cycles were conducted, each consisting of 1 min at 94 °C, 2 min at 37 °C, 3 min of ramp (37–55 °C), 4 min at 55 °C, 30 s at 68 °C. This was followed by a final extension step of 5 min at 68 °C.

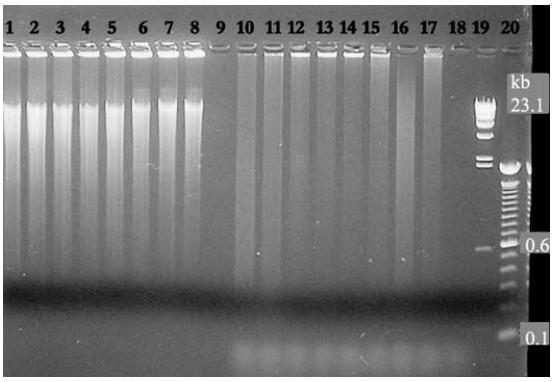

Fig. 1. Agarose gel electrophoresis of WGA products and negative controls (5 μl in each lane). Lanes 1–9: MDA products; lanes 10–18: I-PEP products. Lanes 1–5 and 10–14: products from starting genomic DNA 50, 5, 1, 0.1 and 0.01 ng, respectively. Lanes 6–8 and 15–17: negative controls containing no g-DNA; lanes 6 and 15 contain MDA and I-PEP reagents and H_2O, respectively; lanes 7 and 16 contain only the MDA and I-PEP reagents, respectively; lanes 8 and 17 contain MDA and I-PEP reagents and TE buffer, respectively. Lanes 9 and18: MDA and I-PEP WGA cocktails, respectively, with addition of 50 ng g-DNA, frozen until electrophoresis. Lanes 19 and 20: Lambda DNA/Hind III markers and 100 bp DNA ladder, respectively.

2.2.2. Multiple displacement amplification (MDA)

We have followed the strand displacement method as proposed by Dean et al. [7,8]. The GenomiPhi™ DNA Amplification Kit (Amersham Biosciences) was used to obtain WGA products from 0.01 to 50 ng of genomic DNA in a total reaction volume of 20 μl, which was incubated at 30 °C for 18 h and terminated by heating at 65 °C for 5 min.

2.3. Analysis of short tandem repeat loci

2.3.1. Genotyping protocol

We used the Applied Biosystems kits, AmpFℓSTR® Profiler Plus™ and Cofiler™, which are designed for co-amplification of the 13 CODIS STR loci and the amelogenin locus, which are distributed on 14 different chromosomes. Multiplex PCR amplification of these loci, viz., D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, CSF1PO and the amelogenin locus was conducted in a 25 μl reaction volume containing 0.5–2.5 ng of genomic DNA or WGA products, 9.5 μl of AmpFℓSTR PCR reaction mix, 5 μl of primer set solution and 0.5 μl of AmpliTaq Gold™ DNA