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Comparison of two whole genome amplification methods for STR genotyping of LCN and degraded DNA samples

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Abstract

The analysis of LCN or highly degraded DNA samples presents a challenge for forensic science. Improving the quantity and/or quality of samples would greatly increase the profiling success rate from LCN and degraded samples. Whole genome amplification (WGA) is one method that has such potential. Two commercially available WGA kits, GenomePlex and GenomiPhi, were investigated for use on LCN and degraded DNA samples. Both kits amplified genomic DNA, producing microgram quantities from sub-nanogram templates. Profiling success of LCN DNA samples was increased, with improvements of over 700% from 10 pg template DNA compared to non-WGA-amplified control samples. The amplification success with degraded DNA was also improved by WGA. Degraded DNA was simulated using restriction enzymes to demonstrate that the application of WGA can result in the typing of STR loci that could not previously be amplified. An increase in artefacts, such as stutter alleles and amplification biases, were observed in many samples. Results show that WGA is capable of increasing both the quality and quantity of DNA, and has the potential to improve profiling success from difficult samples in forensic casework.

Keywords: Whole genome amplification; LCN DNA; Degraded DNA; Multiple displacement amplification

1. Introduction

Forensic analysis is often limited by the type and amount of sample available. Samples may be highly degraded or contain only trace amounts of genomic DNA (less than 100 pg). Obtaining complete genetic profiles is difficult from these samples using standard STR amplification methods, and often partial or negative profiles are produced. The use of increased cycle number and other low-copy-number (LCN) techniques can give improved results, from both LCN and degraded templates. However, the resulting profiles may be complex and difficult to interpret, with increased allele imbalance and stutter product formation [1].

Another approach to LCN and degraded DNA profiling yet to be fully examined is the use of whole genome amplification (WGA). Most WGA methods use random primers and low stringency annealing conditions to amplify large sections of the genome to increase the quantity of the starting DNA template, prior to any downstream analysis. Theoretically, WGA is capable of decreasing the stochastic effects resulting from low copy templates [2]. However, it must be capable of replicating the genome with high accuracy and without distorting relative copy number information. The ability to increase the amount of starting template, and/or the quality of the template would be invaluable for forensic applications, provided the product is representative of the original template. The application of WGA to genotyping LCN and degraded samples for forensic use has not been thoroughly examined, particularly in light of the development of several commercial WGA kits.

We investigated two commercial WGA kits to evaluate their ability to amplify LCN and degraded DNA samples. The first, GenomePlex™ WGA (Sigma, St. Louis, MO) is a PCR-based method, involving fragmentation of genomic DNA, following by linker ligation and PCR with universal primers. It is reported to be highly representative, with 99.8% concordance in SNP calls between amplified and genomic DNA. GenomePlex WGA is optimised for use on at least 10 ng of high quality template DNA. The manufacturer reports that single cells and highly degraded DNA are amplified with high accuracy [3], although this has not yet been independently verified.

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In contrast to PCR-based WGA methods, multiple displacement amplification (MDA) replicates the genome isothermally. A novel polymerase, Phi29, replicates the genome exponentially, creating microgram quantities from sub-nanogram template DNA levels. This method produces almost complete genome coverage with little amplification bias and high accuracy [4]. Commercially available MDA kits, such as GenomiPhi™ (GE Biosciences, Piscataway, NJ) used in this study, are optimised for the amplification with a minimum of 1 ng of template DNA, and are not recommended for use with highly degraded templates. Several research groups have, however, demonstrated that MDA is able to accurately amplify single cells [5–7] and very low quantities of genomic DNA [8,9]. It is capable of amplifying DNA directly from a wide range of sample types such as whole blood and buccal cells [10], and the resulting product is reported to be suitable for a wide range of downstream applications, such as STR and SNP genotyping, sequencing and quantitative PCR assays.

We used GenomiPhi and GenomePlex to amplify LCN DNA and degraded DNA to determine if WGA is capable of increasing the quantity and quality of DNA, and produce product suitable for use in a multiplex STR PCR system commonly used in forensic investigations. LCN DNA amplifications were initially performed with buccal cell extracts, to determine if WGA was able to amplify small amounts of high quality template DNA. Casework samples of various origins were also tested to determine the effectiveness of WGA with forensic samples of lower quality. To help determine if WGA was able to provide more complete profiles from degraded DNA we prepared artificially degraded DNA samples. Specifically digested DNA was produced by cleaving genomic DNA with restriction enzymes. This prevented the some of the ten AmpF*l*STR Profiler Plus loci being amplified, but not others, thus simulating highly degraded DNA. WGA was then performed to determine if the digested loci could be recovered. Degraded and inhibited casework samples were also tested.

2. Materials and methods

2.1. DNA samples

Buccal cell samples were chosen based on known genetic profiles of the donors, with high heterozygosity and large size separation between alleles at the loci being examined. Genomic DNA was extracted from buccal swabs with either Chelex® 100 (Bio-Rad Laboratories, Hercules, CA, Ref. [11]) to produce single-stranded DNA, or organic extraction for double-stranded DNA. Buccal samples were quantitated with the Quantifiler Human DNA Quantification system in accordance with the manufacturer's instructions (Applied Biosystems, Foster City, CA). Samples for LCN DNA experiments were diluted to 0.01, 0.05, 0.1, 0.5 or 1 ng/μl in TE. Samples for digested DNA experiments were diluted to 1, 5, 10, 20, 30 or 50 ng/μl in TE. There were four replicates at each template level for each extraction type, and each WGA method.

Non-probative casework samples were also used for GenomiPhi WGA. These samples were from semen, saliva, blood, hair (with apparent sheath material present) and trace origins (6 samples each), as well as degraded and inhibited samples (15 samples), with a range of profiling success rates, and concentrations ranging from 0.015 ng/μl to >1 ng/μl. The degraded DNA samples had adequate DNA quantity (greater than 1 ng), but provided only low partial profiles. The inhibited samples had also had adequate DNA quantity, but only provided negative or low partial profiles, due to the presence of various PCR inhibitors. They were quantified with the Quantifiler Human DNA Quantification system in accordance with the manufacturer's instructions.

2.2. Production of digested DNA

A series of restriction digests were designed to specifically degrade genomic DNA to prevent amplification of specific loci by Profiler Plus. The sequences of the ten Profiler Plus loci were searched for recognition sites using the DNAStar 4.0 MapDraw programme. Four restriction enzymes *HpyCH*4 III (cuts FGA, Amelogenin, D8S1179, D18S51, D5S818), *Mnl* I (D21S11, D18S51, D5S818, D13S317, D7S820), *Bfa* I (vWA, FGA) and *Hph* I (D3S1358, D5S818) were selected which cut in the flanking regions of these repeats, allowing digestion of each locus within the multiplex.

Restriction digests were performed separately overnight with varying amounts of template DNA as stated above, 5 U enzyme (New England Biolabs, Ipswich, MA) and buffer, at 37 °C for 12 h, followed by enzyme inactivation at 80 °C for 20 min. Digestion was confirmed by amplifying 1 μl of the digested sample (ranging from 1 to 50 ng) with the AMPF*l*STR Profiler Plus multiplex (Applied Biosystems). Following genotyping on an ABI 3100 Genetic Analyser and GeneMapper ID, the digest was deemed successful if the target loci were not visible in the electropherogram whilst the non-target loci were visible (see Fig. 3).

2.3. Whole genome amplification

2.3.1. GenomiPhi

MDA WGA was performed with GenomiPhi according to the manufacturer's instructions. Varying amounts of sample DNA in 1 μl were added to 9 μl of GenomiPhi sample buffer, denatured at 95 °C for 3 min, and cooled on ice. Ten microliters of reaction buffer containing dNTPs, random hexamers and Phi29 were added, and the reactions incubated at 30 °C for 16 h. The polymerase was then inactivated by incubation at 65 °C for 10 min. Positive controls with 1 ng of purified human genomic DNA and negative dH_2O controls were performed with each set of reactions. Reaction products were purified by ethanol precipitation, as recommended by the manufacturer.

2.3.2. GenomePlex

GenomePlex WGA was performed according to the manufacturer's instructions. One microliter of sample DNA