TECHNICAL NOTE

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The Utility of Whole Genome Amplification for Typing Compromised Forensic Samples

ABSTRACT: Biological evidence has become invaluable in the crime laboratory; however, it may exist in limited quantity and/or quality. Given this, the ability to amplify total DNA obtained from evidence, in an unbiased manner, would be highly advantageous. Methods for whole genome amplification (WGA) have the potential to fulfill this role, resulting in a virtually unlimited supply of DNA. In the research presented, two WGA methods, improved primer extension preamplification and multiple displacement amplification (MDA), were tested using commercial kits. Control DNA, artificially degraded DNA, and DNA from fresh blood, aged blood, hair shafts, and aged bones underwent WGA, followed by short tandem repeat and mitochondrial DNA analysis. The methods did amplify DNA, but performed poorly on forensically relevant samples; the maximum amplicon size was reduced, and MDA often resulted in extraneous bands following polymerase chain reaction. Taken together, WGA appears to be of limited forensic utility unless the samples are of a very high quality.

KEYWORDS: forensic science, DNA typing, multiple displacement amplification, improved primer extension preamplification, degraded DNA, short tandem repeat, mitochondrial DNA

Over the last two decades, DNA analysis techniques used in forensic science have progressed quickly, requiring less time and ever-smaller amounts of starting material. In spite of this, biological evidence submitted to the laboratory for DNA analysis may still be limiting, a problem exacerbated if a portion of the sample must be maintained for future testing. Concerns associated with limited quantities of DNA could theoretically be alleviated by increasing the overall amount of DNA before beginning standard laboratory analyses, such as short tandem repeat (STR) or mitochondrial DNA (mtDNA) testing. During the past few years, a set of methods capable of replicating the genome in its entirety, termed whole genome amplification (WGA), have been described (1–5). In general, these techniques have been used to amplify pristine clinical samples of high quality that are limited in quantity, although some work on high-quality samples of forensic utility has been conducted (6–8). Research into lower-quality samples, which are likely to be most relevant to forensic applications, are much more limited (9,10). Thus, the utility of WGA for amplification of forensic material, which is often highly compromised, remains unclear.

Two WGA methods that have come into wide use are improved primer extension preamplification (I-PEP (1)) and multiple displacement amplification (MDA (2)). I-PEP is an improved version of the PEP method (5) and utilizes random 15-mers to amplify

DNA, resulting in a reported 30-fold increase in DNA content (1). A commercially available kit (Roche Applied Science, Indianapolis, IN) based on the I-PEP procedure contains two heat-stable polymerases, Taq and Tgo, the latter of which has 3′ exonuclease proofreading activity. This results in an 18-fold lower error rate than Taq alone (Roche product literature). MDA, on the other hand, utilizes the highly processive polymerase Phi 29, which can generate products as large as 70 kb, although the average size is around 10 kb (11). Phi 29 is utilized at a single temperature (30°C), and also has proofreading activity (2). The enzyme displaces double-stranded DNA ahead of it, with the new single-stranded DNAs representing additional targets to which primers (random hexamers) can anneal. It has been reported that DNA amplification up to 10,000-fold can be achieved using MDA (12).

The goal of the research presented here was to characterize these two WGA methods for use on samples commonly encountered in forensic casework. This included determining the usefulness of WGA when DNA quantities were limited, and/or when DNA quality was poor. I-PEP and MDA were first optimized on high-molecular-weight DNA. Next, the same DNA was artificially degraded and separated into size class, to examine how the length of pre-WGA DNA influenced subsequent nuclear and mtDNA analyses. Finally, DNAs from forensically informative samples, including fresh human blood, aged human blood, human hair shafts, and human bone, were whole genome amplified and the resultant products were tested.

Materials and Methods

WGA Optimization

I-PEP was performed using an Expand High Fidelity PCR system (Roche Applied Science) with random 15-mers produced

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in-house. MDA was performed with a GenomiPhi DNA amplification kit (Amersham Biosciences/GE Healthcare, Piscataway, NJ). Protocols from (1) for I-PEP and from the manufacturer for MDA were followed, amplifying samples in 10 μL reaction volumes. Following WGA, excess primers and nucleotides were removed via filtration through a Microcon YM-100 column (Millipore Corporation, Bedford, MA). Both WGA techniques were first tested using 100, 10, and 1 pg of high-molecular-weight control DNA (Promega, Madison, WI), as well as no-DNA (negative) controls. For some experiments (e.g., STR analyses) single source male (buccal) DNA, produced in-house, was utilized.

Preparation of Artificially Degraded and Forensically Relevant DNAs

Control DNA was randomly degraded using DNase I (Promega). Following preliminary experiments testing the levels of digestion, 1.5 μg of DNA was subjected to 0.75 U of DNase I at 17°C in a final reaction volume of 30 μL. DNase activity was stopped after 0, 30, 60, and 120 s by transferring 1/4 of the reaction to a tube containing 2.5 μL of 20mM ethylenediaminetetraaceticacid (EDTA) (13). The 30–120 s reactions were combined, denatured at 95°C for 5 min, chilled on ice, and electrophoresed on a 2% low melting point agarose gel, adjacent to a 100 base sizing ladder (gel comparison of denatured and nondenatured size standard showed very similar migration patterns; thus for simplicity, the nondenatured ladder was used). Sections of gel corresponding to < 200, 200–400, 400–600, 600–800, 800–1000, and > 1000 nucleotides were cut from the gel, placed in 1.5 mL microcentrifuge tubes, heated to 65°C, and organically extracted (phenol, followed by chloroform). DNAs were precipitated using 1/10 the volume of 3 M sodium acetate and 2 volumes of 95% ethanol. DNA pellets were vacuum dried and resuspended in 12.5 μL of TE, resulting in an *c*.10 ng/μL stock (estimating a one-third loss of DNA during gel isolation and purification). Two nanograms of degraded DNA was used for subsequent WGA reactions.

Blood DNAs were prepared by placing 1 μL of fresh human blood into a microcentrifuge tube and allowing it to dry overnight (larger volumes were also prepared, but were not needed for subsequent WGA experiments). An *c*.1 mm^2 section of dried human blood that had been aged at room temperature in a dark, dry environment for 16 months, was also prepared. Blood samples were resuspended in 300 μL of digestion buffer (20 mM Tris, 100 mM EDTA, 0.1% sodium dodecyl sulfate) along with 3 μL of 20 mg/mL proteinase K, and incubated for 1 h at 55°C. The samples were organically extracted and precipitated as described above. DNAs were resuspended in 15 μL of tris-EDTA (TE), and 1 μL was used for WGA.

DNAs from human hair shafts were obtained using a standard manual grinding/organic extraction technique, and have been previously described (14). Skeletal samples came from two sources: a Pittsburgh area cemetery dating to the early/mid 1800s (15,16), and a burial mound near the village of Kamenica, Albania, dating from 3000 to 2500 years before present (17). These were also prepared through organic extraction, followed by purification using Microcon YM-100 filtration. Based on previously successful amplification of mtDNA from these samples, 1 μL of a 1:20 dilution of the hair DNAs, 1 μL of a 1:10 dilution of the Pittsburgh skeletal DNAs, and 1 μL of the Albania skeletal DNAs were used for WGA. Hair and bone reactions included 1 μL of 3 μg/μL bovine serum albumin (BSA), as this had resulted in earlier successful PCR attempts.

Assaying Post-WGA Products

A variety of methods was used to examine post-WGA DNAs, including testing the quantity of DNA produced relative to the amount of DNA existing in the original sample (i.e., the overall level of WGA), the maximum size of nuclear and mitochondrial amplicons that could be generated following WGA (again, compared with pre-WGA DNA from the same samples), and the quality of post-WGA PCR products (genotypes consistent with pre-WGA DNA, clean amplification products, etc.).

WGA success and molecular weights of post-WGA total DNA were examined through electrophoresis of post-WGA DNAs on 1% agarose gels. Next, pre- and post-WGA DNAs were serially diluted until PCR amplification of nuclear or mitochondrial loci was no longer successful (ranging from 10 to 1,000,000-fold). Dilutions of post-WGA DNA that resulted in a single, strong target band were used for subsequent PCR reactions (testing of larger amplicons or multiplexes). If these were unsuccessful, greater quantities of post-WGA DNA were also tested.

Primers and resultant amplicon size for the single copy nuclear locus, amelogenin, and regions of the mtDNA control region are displayed in Table 1. All reactions contained 1 × Hot Start PCR buffer (Eppendorf, Westbury, NY), 0.2 mM dNTP, 2 μmol of each primer, and 1 U HotMaster Taq (Eppendorf) in a final reaction volume of 10 μL, or 20 μL if the product was to be sequenced. BSA was added to some samples as described above. The cycling parameters for amelogenin amplification were 94°C for 2 min; 38 cycles of 94°C for 1 min, 58°C for 45 s, and 72°C for 45 s. Cycling parameters for mtDNA amplification were 94°C for 2 min, followed by 38 amplification cycles of 94°C for 30 s, 56°C for 45 s, and 72°C for 45 s. A final extension of 72°C for 5 min was added at the end of all amplifications.

TABLE 1—*PCR primers and amplicons.*

Primer Name	Primer Sequence
F Amel	5'-CTCCCCTCCTCCCTGTAAAA-3'
R Amel 213	5'-TAAACTGGGAAGCTGGTGGT-3'
R Amel 496	5'-AGCAGAGGCAAGCAAGAGAC-3'
F15	5'-CACCCTATTAACCACTCACG-3'
F82	5'-ATAGCATTGCGAGACGCTGG-3'
F155	5'-TATTTATCGCACCTACGTTC-3'
F15989	5'-CCCAAAGCTAAGATTCTAAT-3'
F16190	5'-CCCCATGCTTACAAGCAAGT-3'
R285	5'-GTTATGATGTCTGTGTGGAA-3'
R484	5'-TGAGATTAGTAGTATGGGAG-3'
R16144	5'-TGACCACCTGTAGTACATAA-3'
R16410	5'-GAGGATGGTGGTCAAGGGGAC-3'

Primer Pair	Amplicon Length (bp)
F Amel/R Amel 213	213
F Amel/R Amel 496	496
F82/R285	203
F16190/R16410	220
F15/R285	270
F155/R484	329
F82/R484	402
F15989/R16410	421
F16190/R285	664
F15989/R285	865
F15989/R484	1064

PCR primer sequences utilized for amelogenin (Amel) and mtDNA amplification. F, forward primer; R, reverse primer. Amelogenin numbers indicate the amplicon size produced, while the mitochondrial numbers show the primer position relative to the reference sequence (19). Primer pair specifies the combination of primers utilized, followed by the size of the amplicon produced in base pairs (bp).

STR analysis was conducted using an Identifiler kit (Applied Biosystems, Foster City, CA) following the manufacturer's recommendations, with reactions scaled down to 10 µL and amplification cycle number increased to 35. Capillary electrophoresis was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Data were analyzed with ABI GeneMapper ID, version 3.1. Quantities of post-WGA DNA added to the reactions were based on levels that produced quality results with the single-locus assay(s); samples that did not generate usable results during single-locus testing were not amplified using the multi-locus kit.

MtDNA amplicons were purified on a Montage PCR Centrifugal Filter Device (Millipore), with one 400 µL rinse of TE and centrifugation at $1000 \times g$. Sequencing was performed using a CEQ DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA) as instructed by the manufacturer. Sequences were analyzed on a Beckman Coulter CEQ 8000 Genetic Analysis System, and aligned using Bio Edit software (18) and the Anderson reference sequence (19). Sequence results were ultimately compared with those previously obtained (14,16,17).

Results

The characterization of I-PEP and MDA was undertaken using a variety of samples, ranging from those with large quantities of high-molecular-weight DNA to samples with low quantities of heavily degraded DNA. Whole genome amplified products were compared with untreated DNA in terms of quantity (how far a post-WGA sample could be diluted and still generate a PCR product), quality (whether there was a change in the maximum obtainable PCR amplicon size), and the success of downstream analyses, including mtDNA sequencing and examination of STRs. Fig. 1 displays total DNA smears resulting from preliminary tests of both WGA
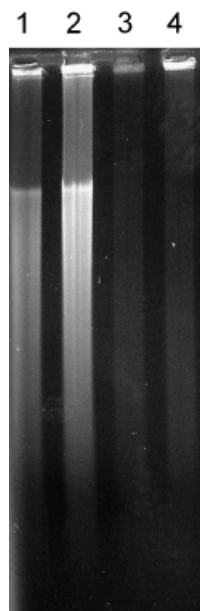

FIG. 1—*Whole genome amplified (WGA) products. Post-WGA DNA was separated on a 1% agarose gel. Lane 1—multiple displacement amplification (MDA) product containing no starting genomic DNA. Lane 2—MDA product resulting from 1 ng of size fractionated DNA >1000 nucleotides. Lane 3—improved primer extension preamplification (I-PEP) product containing no starting genomic DNA. Lane 4—I-PEP product resulting from 1 ng of size fractionated DNA >1000 nucleotides. Note the high-molecular-weight smears in all lanes, including one and three that only contained random 6-mers or 15-mers, respectively, as starting nucleic acids.*

methods on size-fractionated DNA of >1000 nucleotides (equivalent to 1 ng of starting material), as well as negative controls. Both methods increased the quantity of DNA, and as reported (2,20), MDA produced far more product than I-PEP. Somewhat surprisingly, both methods generated large quantities of high-molecular-weight product in the negative controls, which contained only primers and free nucleotides (no human DNA). The same was seen when highly degraded DNA was used as a starting template. This made simple agarose gel electrophoresis (or any method that assayed overall DNA yield) unsuitable for assessing the success of the WGA procedures; thus allele-specific, PCR-based techniques were utilized.

WGA of High-Quality Samples

Both WGA methods were useful in amplifying high-molecular-weight control DNA, as well as DNA equivalent to 1/15th µL of fresh blood and blood aged for 16 months. Before WGA, the largest amplicons examined, 496 bp for amelogenin and 1064 bp for mtDNA, were successfully amplified, the lone exception being the aged blood, from which mtDNA could only be amplified at 865 bp. Following I-PEP and MDA, the largest amplicons were obtained from all samples.

Based on these results, the level (amount) of amplification was measured through DNA dilution followed by assaying the 496 bp nuclear amplicon. DNA from fresh blood amplified at a 10-fold dilution, while I-PEP-treated DNA amplified at a 10,000-fold dilution, and MDA-treated DNA at a 100,000-fold dilution (or a 1000- and 10,000-fold increase, respectively). Pre-WGA DNA from aged blood could only be amplified using undiluted sample, while following I-PEP and MDA it could be diluted 100-and 1000-fold, respectively. Amplifications generated a single band of the appropriate size (data not shown), except from the post-MDA aged blood DNA, in which the target amelogenin band was present, but the reactions also contained a large number of extraneous bands (see examples in MDA results presented below).

STR analysis was undertaken on the same samples, where again a 10-fold dilution of pre-WGA DNA from fresh blood produced a full profile. I-PEP amplified DNA from fresh blood also produced a full profile, although it required 1 µL of undiluted WGA product. MDA product from fresh blood also required more DNA, with the 1000-fold diluted sample matching at 14 of 16 loci (two showed allelic dropout; higher DNA concentrations were not tested). In contrast, no STR profiles could be generated from untreated-aged blood samples, while I-PEP DNA matched at two loci (D19S433 and amelogenin, which are both short amplicons), and two additional loci showed allele dropout (D8S1179 and D5S818; also short amplicons). Aged blood DNA that underwent MDA gave a partial profile at one locus (D8S1179).

WGA of DNase I-Degraded DNA

Nuclear and mtDNA amplification results from artificially degraded DNA trials are shown in Table 2. The 496 bp nuclear DNA target was not amplifiable from pre-WGA DNA of any size class, and was obtained in two of 12 I-PEP samples (owing to lack of success, it was not tested in trial 3). The 213 bp nuclear target was amplifiable in 13 of 18 pre-WGA samples, with some variability among size classes. The results were improved slightly following I-PEP (15 of 18 samples were positive), although in two instances samples that were positive before I-PEP were negative following it. The results were consistent for mtDNA, in that larger amplicons could not be recovered before or after I-PEP, with maximum amplicon sizes being 220 or 270 bp. In four instances, the 270 bp

TABLE 2—*Amplification of size-fractionated DNA.*

	Trial 1			Trial 2			Trial 3			Summary Data		
	No WGA	I-PEP	MDA	No WGA	I-PEP	MDA	No WGA	I-PEP	MDA	No WGA	I-PEP	MDA
<200												
Nuc-213 bp	+	−	−	−	−	[−]	−	+	−	1/3	1/3	0/3
Nuc-496 bp	−	−	−	−	−	−	ND	ND	ND	0/2	0/2	0/2
Largest mt	220	220	[220]	220	220	[220]	270	270	ND			
200–400												
Nuc-213 bp	+	+	+	+	−	−	+	+	−	3/3	2/3	1/3
Nuc-496 bp	−	−	−	−	−	−	ND	ND	ND	0/2	0/2	0/2
Largest mt	270	270	[220]	220	220	[220]	270	270	ND			
400–600												
Nuc-213 bp	+	+	+	+	+	[−]	+	+	−	3/3	3/3	1/3
Nuc-496 bp	−	−	−	−	−	−	ND	ND	ND	0/2	0/2	0/2
Largest mt	270	270	[220]	220	270	[220]	270	270	ND			
600–800												
Nuc-213 bp	−	+	+	+	+	[−]	+	+	−	2/3	3/3	1/3
Nuc-496 bp	−	−	−	−	+	−	ND	ND	ND	0/2	1/2	0/2
Largest mt	220	220	[220]	220	270	[220]	270	270	ND			
800–1000												
Nuc-213 bp	−	+	−	−	+	+	+	+	−	1/3	3/3	1/3
Nuc-496 bp	−	−	−	−	+	−	ND	ND	ND	0/2	1/2	0/2
Largest mt	270	270	[220]	220	270	[220]	270	270	ND			
>1000												
Nuc-213 bp	+	+	−	+	+	+	+	+	−	3/3	3/3	1/3
Nuc-496 bp	−	−	−	−	−	−	ND	ND	ND	0/2	0/2	0/2
Largest mt	270	270	[220]	220	270	[220]	270	270	ND			

Three trials of nuclear and mitochondrial PCR amplification before and after WGA techniques. The *Y*-axis displays the DNA size classes (in bases) tested, while the *X*-axis displays results from the three trials, summary data, and treatments (no whole genome amplification, I-PEP, and MDA). "Nuc" indicates nuclear (amelogenin) amplification, while "mt" indicates mitochondrial DNA amplification. +indicates positive, clean results, [ ] around a size class indicates that both the target and extraneous bands were generated, and [−] indicates only extraneous bands were produced. ND, not determined; WGA, whole genome amplification; MDA, multiple displacement amplification; I-PEP, improved primer extension preamplification.

amplicon was generated after I-PEP, while only the 220 bp product was obtained before WGA.

MDA results from artificially degraded DNA were generally less clean. As was seen in aged blood samples, while the target amplicon was often present, multiple extraneous bands were also produced (Fig. 2*a*). The 496 bp nuclear product was once again not obtained, while a single 213 bp band was generated from five of 18 samples, and three others had several extraneous bands. A clean mitochondrial product was not obtained from any size class in the first two MDA trials, and thus was not tested in trial 3. The extraneous bands generally varied among size classes, but were consistent among PCR reactions within a size class (Fig. 2*b*).

STR analysis from pre-WGA artificially degraded DNA (trial 1) produced results superior to those post-WGA in all cases. Pre-WGA DNAs in size classes 400–600, 600–800, 800–1000, and >1000 nucleotides matched the known profile at 14, 16, 15, and 16 loci, respectively. Correct results from the 200–400 size class occurred at seven loci, partial profiles were obtained at three loci, four loci could not be called due to multiple peaks, and two were negative. The <200 size class from pre-WGA DNA generated no correct allele calls. Following WGA, no STR profiles from fractions <1000 nucleotides were generated. I-PEP DNA from the >1000 nucleotide fraction matched the known profile at four loci, and a partial profile was generated at seven additional loci. MDA DNA from the >1000 nucleotide fraction matched at nine loci, and a partial profile was obtained at two loci. In virtually all cases the larger amplicons gave the negative results.

WGA of Head Hair Shaft and Aged Skeletal DNA

WGA was attempted on DNA isolated from head hair shafts and aged skeletal remains that had previously been shown to con-tain amplifiable mtDNA (14,16,17). DNA volumes used for WGA were the same as had been used for successful mtDNA amplification. The five hair shaft samples amplified using I-PEP were
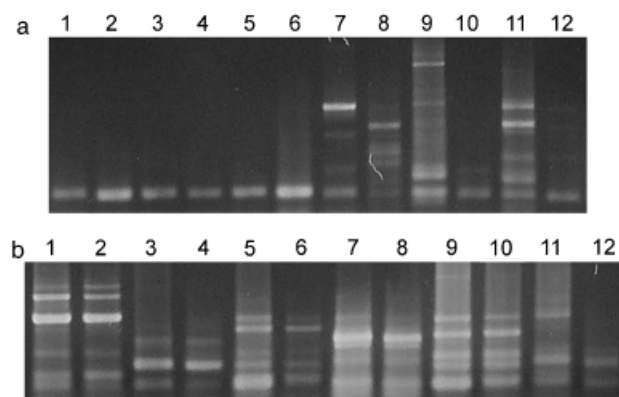

FIG. 2—*Post whole genome amplification product from artificially degraded control DNA. 2a—Amplification of a 220 bp mtDNA fragment following improved primer extension preamplification (I-PEP) (lanes 1–6) and multiple displacement amplification (MDA) (lanes 7–12). The <200 (lanes 1 and 7), 200–400 (lanes 2 and 8), 400–600 (lanes 3 and 9), 600–800 (lanes 4 and 10), 800–1000 (lanes 5 and 11), and >1000 (lanes 6 and 12) nucleotide size classes are shown. The correct amplicon was produced following the I-PEP procedure, while multiple extraneous bands were generated following MDA. 2b—Post-MDA PCR amplification of a 220 bp mtDNA fragment from the <200 (lanes 1 and 2), 200–400 (lanes 3 and 4), 400–600 (lanes 5 and 6), 600–800 (lanes 7 and 8), 800–1000 (lanes 9 and 10) and >1000 (lanes 11 and 12) nucleotide size classes, using 1 µL neat (odd numbered lanes) and 10-fold diluted (even numbered lanes) of DNA. Note that the extraneous bands generated from each size class were different from one another, but separate amplifications of any one size class generated the same extraneous bands, indicating that these arise as part of the WGA process.*

successfully amplified, and generated a 20–2000-fold increase in mtDNA levels based on serial dilution and successful mtDNA amplification. Before WGA, these had a maximum mtDNA amplicon size of 865 bp (14). After I-PEP, the maximum mtDNA PCR product length was reduced for all: DNA from two samples had a maximum amplicon length of 664 bp, one had a maximum amplicon length of 402 bp, and two had a maximum amplicon length of 203 bp. Subsequent sequencing of the mtDNA products showed that all were consistent with the original hairs (the same polymorphisms existed), although I-PEP samples displayed ambiguous (double) peaks at three locations that were cleanly called before WGA. MDA-treated hair samples were far less successfully amplified, once again generating a large number of nonspecific bands following mtDNA PCR, which were not useful for further analysis. In no instance was nuclear DNA successfully amplified following WGA of hair shaft DNA.

Aged (150–3000 years) skeletal material from which mtDNA had previously been amplified (16,17) produced inferior results following WGA. After I-PEP, a single bone sample generated mtDNA data (from the youngest material), while MDA treatment again resulted in a large number of nontarget bands. Given this, nuclear DNA testing was not attempted.

Discussion

Both WGA methods produced large amounts of product when high-molecular-weight DNA (positive control or fresh blood) was amplified, with MDA producing more product than I-PEP (e.g., Fig. 1). Based on product dilution and subsequent PCR, MDA amplified the DNA up to 10,000-fold, while I-PEP generally amplified DNA 1000–2000-fold. Good STR profiles were obtained from these samples as well, and the maximum nuclear and mitochondrial amplicon sizes tested were generated. However, both methods also produced high-molecular-weight product even when no input DNA was included, presumably resulting from the random primers repeatedly pairing and extending. Such an outcome has been alluded to in the literature (2,21), and is of particular concern for forensic applications, where samples may be encountered that contain little or no target DNA—the precise types of samples that might benefit from WGA. The random nature of WGA also means that any exogenous DNA, including microbial material that would be expected with recovered remains, will also amplify, and may in fact swamp out successful amplification of the target DNA.

The two WGA methods produced quite different results on suboptimal samples when compared with high-quality samples. Artificially degraded control DNA, as well as material likely to be encountered in the forensic laboratory (aged blood, hair shafts, and bone), always contained large amounts of high-molecular-weight product following WGA, but its analysis was far less likely to be successful. The best results from forensic samples were obtained from aged blood, which was amplified c. 100- and 1000-fold with I-PEP and MDA, respectively. Before WGA, STR profiles were not obtained from the aged blood DNA, while following WGA a limited number of loci could be amplified. However, these represented only the smallest amplicons in the Identifiler multiplex, and even among these, allelic dropout occurred. In contrast, all but the smallest size classes of artificially degraded DNA produced usable STR profiles pre-WGA, but none yielded useful data post-WGA. Similarly, nuclear DNA could not be amplified from pre- or post-WGA hair shafts, nor from the skeletal material; thus, there was no improvement in nuclear DNA analysis after WGA. I-PEP treatment did increase hair mtDNA levels 20–2000-fold,

although the maximum amplicon size was reduced. MtDNA from aged skeletal material, successfully amplified pre-WGA, could not be amplified post-WGA in all but one instance (an I-PEP sample). Likewise, in the artificially degraded DNA only the smallest nuclear and mitochondrial amplicons were amplifiable following WGA, even from the largest size classes (Table 2).

These results raise an obvious concern about the application of WGA in forensic investigations. The major utility of WGA on forensic samples will often be on evidentiary material of poor quality, and this is where WGA appears most likely to fail. The reason for this seems clear—resulting from the random amplification nature of the methods. An amplification procedure that uses random primers to increase DNA quantity will necessarily reduce DNA fragment size as it progresses. Any given primer is statistically unlikely to anneal at the 3′-most end of a target molecule, and instead, will anneal randomly along its entire length. The resultant product pool, while covering the range of possible amplicon sizes (from full length [3′ binding] to no added bases [5′ binding]), will average a 50% reduction in size compared with the starting material, and of course, have a resultant loss of desired PCR primer sites. Subsequent rounds will continue the trend in size reduction. While this situation does not appear to be a concern when high-molecular-weight DNA is available, quite the opposite is true of degraded material. Low-molecular-weight DNA that may be difficult to amplify already, including that found in many forensic samples, can only generate smaller and perhaps unamplifiable product. As seen in the experiments conducted here, all but the largest size classes of the post-WGA artificially degraded DNA did not produce nuclear/STR profiles, nor did the forensic samples. MtDNA amplification was also greatly reduced in success rate and amplicon size.

A second concern for these methods stems from the production of extraneous amplicons following WGA. I-PEP tended to produce "cleaner" results than MDA (also observed in (6)), which was particularly true for degraded material. Multiple bands (often including the target amplicon) were seen in almost all post-MDA PCR products originating from forensic samples and artificially degraded DNA, precluding subsequent analyses and thus producing fruitless results (Fig. 2a). The exact reason for this is uncertain, but most likely stems from the prolific DNA amplification that Phi 29 generates, even when little or no target DNA is placed in the reaction (Fig. 1). Interestingly, the numerous nontarget bands produced following post-MDA nuclear and mitochondrial amplification showed no regular pattern among samples, including those originating from the same starting DNA, such as the artificially degraded samples (Fig. 2b). In contrast, the nontarget bands were generally quite reproducible within a sample (Fig. 2b), indicating that it was the degraded sample itself, and not random amplification, that resulted in the bands seen.

Conclusions

Taken together, the results presented here indicate that WGA, and more specifically the two commercially available methods tested, may be of limited utility for analyzing degraded/forensically relevant samples, which represent the exact types of material that would call for this methodology. The overall size reduction of DNA during WGA—with the molecular weight necessarily decreasing with every round—means that degraded DNA, which already has a susceptibility to amplification failure and allelic dropout, is likely to become worse post-WGA, as will its successful analysis. Further, the random products that WGA (and in particular the MDA procedure) generate from limited (or no)

starting material can lead to the creation of multiple nonspecific PCR amplicons, further hampering downstream analyses. While WGA appears to work well on high-quality DNA samples, its use in forensic analyses, which often combine low-quality DNA with limited material, may be restricted. An exception to this could be a common forensic sample not tested in the experiments presented here—swabs taken following sexual assaults. These may indeed represent instances where DNA is limiting, but is still in good enough condition that WGA could be useful. More research on assault samples needs to be conducted.

Regardless of these potential outcomes, commercially available kits designed for nonforensic material will need to be retooled if they are to have general applicability in forensic investigations. Further, the type of sample amplified, and more importantly the quality of the DNA within, are important factors that must be considered before WGA. This is not to say that WGA will never be a viable technique for forensic scientists, but it does not yet appear to be a useful method for amplifying the varied and often unpredictable material that enters the forensic DNA laboratory.

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