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# Modified DOP-PCR for improved STR typing of degraded DNA from human skeletal remains and bloodstains

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ABSTRACT

Forensic and ancient DNA samples often are damaged and in limited quantity as a result of exposure to harsh environments and the passage of time. Several strategies have been proposed to address the challenges posed by degraded and low copy templates, including a PCR based whole genome amplification method called degenerate oligonucleotide-primed PCR (DOP-PCR). This study assessed the efficacy of four modified versions of the original DOP-PCR primer that retain at least a portion of the 5′ defined sequence and alter the number of bases on the 3′ end. The use of each of the four modified primers resulted in improved STR profiles from environmentally-damaged bloodstains, contemporary human skeletal remains, American Civil War era bone samples, and skeletal remains of WWII soldiers over those obtained by previously described DOP-PCR methods and routine STR typing. Additionally, the modified DOP-PCR procedure allows for a larger volume of DNA extract to be used, reducing the need to concentrate the sample and thus mitigating the effects of concurrent concentration of inhibitors.

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## 1. Introduction

The robustness and reliability of forensic STR analyses are directly correlated to the quantity and quality of the DNA available for testing. Samples containing degraded and/or low-copy number (LCN) templates can be particularly problematic. An increase in the number of viable template molecules for amplification of DNA may enhance chances of obtaining results from such challenged samples. One approach to increase viable template molecules is DNA repair which focuses on restoring fragmented or otherwise degraded DNA, although with limited success [1].

Whole genome amplification (WGA) represents an alternative approach for potentially improving the success of STR typing from degraded and/or low-copy templates. WGA can be particularly relevant in forensic and ancient DNA analyses, where availability of sufficient quantities of DNA is critical for the success of STR genotyping and other downstream applications. While early WGA methodologies were used primarily on limited quantity clinical specimens for medical diagnostics, genetic testing, and genomic research, interest in the applicability of these methods to forensic analyses has increased for improving the possibility of obtaining genetic data from degraded/LCN samples.

WGA methods were first described in the early 1990s [2–6], and a variety of approaches has emerged. There essentially are two categories of WGA: multiple displacement amplification (MDA) and methods involving variations of PCR [2–15]. MDA has been shown to produce complete genomic DNA amplification with low amplification bias. The high fidelity of the ϕ29 DNA polymerase used in MDA results in accurate genotyping [4,9]. However, the success of MDA is highly dependent on the starting quantity and quality of DNA template used in the reaction, which limits the applicability of this method with the types of samples typically encountered in forensic casework. MDA protocols and commercially-available MDA kits (GenomePlex®, GenomiPhi®) recommend input quantities of DNA in the 10–100 ng range and are tolerant to mild-to-moderate DNA degradation. It requires high-quality, high molecular weight DNA (usually >2 kb) to be successful [7]; therefore, moderate-to-severely degraded DNA negatively impacts MDA efficiency [4,7,9,11,16].

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In contrast, PCR-based WGA methods are affected less by DNA quantity and quality, and thus hold more potential as a tool for working with LCN and degraded templates [2–3,7,13,15,17–18]. One promising PCR-based WGA method is degenerate-oligonucleotide-primed PCR (DOP-PCR). DOP-PCR, first described in 1992, provided the capability of efficiently amplifying relatively short DNA templates and yielded microgram quantities of genome-representative DNA from picogram or nanogram amounts of starting material [2]. In contrast to the pairs of target-specific primers used in traditional PCR, only a single primer is used in DOP-PCR. The originally reported DOP-PCR primer (5′-CCGACTCGAGNNNNNNATGTGG-3′) had defined sequences at both the 5′ and 3′ ends, with an internal random hexamer sequence. The 10-bp defined sequence at the 5′ end of the oligonucleotide contained a 6-bp *XhoI* restriction site that was originally incorporated for use in downstream cloning experiments [2–3,12–13].

The defined sequences at both the 5′ and 3′ ends of the DOP-PCR primer were important for efficient and successful WGA [2]. The original DOP-PCR method was comprised of two separate cycling stages, a low-stringency followed by a high-stringency reaction. Initial low-stringency cycles ensured annealing of the 6-bp 3′ defined sequence to approximately $10^6$ complementary sites in the human genome. The adjacent random hexamer sequence contains all possible combinations of dNTPs so that the primer could anneal to various sites on the template DNA to initiate synthesis during the DOP-PCR. The 10-bp 5′ defined sequence reportedly permitted efficient annealing of primers to previously-amplified DNA, allowing a higher annealing temperature to be used in subsequent (high-stringency) PCR cycles [2–3,12–13].

Bonnette et al. [17] and Dawson Cruz [18] investigated the effects of increasing the degeneracy of the original (6N) DOP-PCR primer to 10N and 16N, by removing the first 4 bp of the 5′ defined sequence (leaving only the *XhoI* restriction site) and by completely removing the 10-bp 5′ defined sequence, respectively. Results demonstrated that both the 10N and 16N primers outperformed the original 6N primer in terms of improving the quality of STR profiles obtained from low-copy and degraded samples. However, given the above assertion that the 5′ defined sequence is crucial for efficient annealing of the primer to low-stringency DOP-PCR WGA products, and because downstream cloning experiments are not a typical part of processing forensic casework samples, other primer designs may be more efficient. The study herein assessed the efficacy of four modified versions of the original DOP-PCR primer that retain at least a portion of the 5′ defined sequence and alter the number of bases on the 3′ end. The efficacy of the modified primers was evaluated by improvement of STR typing of degraded and LCN samples.

## 2. Materials and methods

### 2.1. Human cell line DNA

Female (9947A) and male (007) human cell line DNA were obtained from the AmpFlSTR® Identifiler® Plus and AmpFlSTR® Yfiler® PCR Amplification Kits, respectively (Life Technologies, Foster City, CA).

### 2.2. Degraded/compromised samples

Whole human blood samples were environmentally-damaged as described in [1]. All samples were anonymized and collected in accordance with methods approved by the Institutional Review Board of the University of North Texas Health Science Center in Fort Worth, Texas USA.

Contemporary skeletal remains consisted of 1 femur and 1 tibia from two different individuals. Historical bone samples included the 120-year-old skeletal remains (right femur, both tibiae, four teeth) of an exhumed American Civil War soldier [1,19] and the skeletal remains (femora and tibiae) of four Finnish World War II soldiers (provided by the Department of Forensic Medicine, University of Helsinki, Helsinki, Finland) [20].

### 2.3. DNA extraction

Skeletal remains were extracted as described in Ambers et al. [1,19]. Whole human blood samples were extracted using the QIAamp DNA Investigator Kit (Qiagen, Valencia, CA).

### 2.4. DNA quantification

The quantity of DNA from all extracts was determined using the Quantifiler® Human DNA Quantification Kit (Life Technologies, Foster City, CA), according to the manufacturer's recommendations.

### 2.5. Primer degeneracy

Seven different DOP-PCR primers (six modified and the original published primer) were investigated. The original DOP-PCR primer was modified by removing the unnecessary restriction site and reducing the required bases on the 3′ end of the primer. Table 1 lists the degenerate primers used in the DOP-PCRs, including the original DOP-PCR primer (6N), two primers (10N dcDOP and 16N dcDOP) from a study by Dawson Cruz [18], and four newly-modified primers (abDOP) that retain at least a portion of the 5′ defined sequence and alter the number of bases on the 3′ end. The primer designations "dcDOP" and "abDOP" reflect modifications made to the DOP primer by Dawson Cruz (using the prefix "dc") [18] and the ones designed in this study, respectively (with the "ab" prefix referring to the first two letters of the alphabet just to differentiate this first iteration of novel primers).

### 2.6. DOP-PCR master mix preparation

The DOP-PCR master mix was based on the original Roche DOP-PCR Master Kit (Roche Molecular, Mannheim, Germany). Per sample, the master mix consisted of 10 μl of 10× High Fidelity PCR Buffer (Invitrogen), 4.0 μl of 50 mM $MgSO_4$, 5.0 μl of dNTPs (4 mM each), 5.0 μl of degenerate primer (40 μM), and 0.5 μl of

**Table 1**
Primers used for DOP-PCR. The portion of the 5′ defined sequence in **bold** (**CTCGAG**) represents the original *XhoI* restriction site for cloning.

| Primer name | Primer sequence | Primer description |
|---|---|---|
| 6N DOP | 5′-CCGA**CTCGAG**NNNNNNATGTGG-3′ | Original DOP-PCR primer [2] |
| 10N dcDOP | 5′-**CTCGAG**NNNNNNNNNNATGTGG-3′ | Modified dcDOP-PCR primer [17–18] |
| 16N dcDOP | 5′-NNNNNNNNNNNNNNNNATGTGG-3′ | Modified dcDOP-PCR primer [17–18] |
| 10N abDOP | 5′-CCGA**CT**NNNNNNNNNNATGTGG-3′ | CT from *XhoI* restriction site remaining |
| 12N abDOP | 5′-CCGANNNNNNNNNNNNATGTGG-3′ | Complete removal of *XhoI* restriction site |
| 12N(2) abDOP | 5′-CCGA**CT**NNNNNNNNNNNNGTGG-3′ | CT from *XhoI* restriction site remaining; Shortened 3′ sequence from 6 bp to 4 bp |
| 14N abDOP | 5′-CCGANNNNNNNNNNNNNNGTGG-3′ | Complete removal of *XhoI* restriction site; Shortened 3′ sequence from 6 bp to 4 bp |

Platinum *Taq* High Fidelity DNA Polymerase 5 U/μl (Invitrogen, Carlsbad, CA). Using sterile filter tips, 24.5 μl of master mix were added to each sample tube, and after addition of 1–50 μl of degraded or LCN template, 25.5–74.5 μl of $TE^{-4}$ buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) were added to bring the total reaction volume up to 100 μl. Five microliters of 9947A control DNA (0.1 ng/μl) and 5 μl of $TE^{-4}$ buffer served as positive and negative controls, respectively. Quantities of 100 pg and 500 pg of both 9947A (female) and 007 (male) control DNA were amplified separately using each of the seven degenerate primers. For degraded and LCN samples, a range of input DNA template amounts (100 pg to 1 ng) was explored to determine the minimum and maximum quantity needed for optimal DOP-PCR results.

#### 2.6.1. Original DOP-PCR amplification method

Amplification of the 100 μl reaction mixture was carried using the ABI GeneAmp® 9700 PCR System (Life Technologies, Foster City, CA), with the following PCR parameters: initial incubation at 95 °C for 5 min; 5 cycles of non-specific amplification (94 °C for 1 min, 30 °C for 1.5 min, and 72 °C for 3 min) with a 3-min ramp to 72 °C; 35 cycles of specific amplification (94 °C for 1 min, 62 °C for 1 min, and 72 °C for 3 min) with a 1-s increase in each subsequent cycle; and a final extension at 72 °C for 10 min.

#### 2.6.2. Amplification method for modified dcDOP and abDOP primers

Samples (100 μl total reaction volume) were amplified with the ABI GeneAmp® 9700 PCR System (Life Technologies, Foster City, CA), as described by Dawson Cruz [18]. This method used for this study increased the number of non-specific amplification cycles to twelve (as opposed to the five low-stringency cycles used in the original DOP-PCR) [2,18].

### 2.7. Sample concentration after DOP-PCR

Following DOP-PCR amplification, all samples were concentrated using Amicon® Ultra-0.5 centrifugal filter units with Ultracel-10 membranes (Millipore, Billerica, MA). After prehydrating the membrane of the filter unit with 25 μl of molecular grade $H_2O$, the entire volume of DOP-PCR product (100 μl) and an additional 375 μl of molecular grade water were added to the Amicon® (500 μl maximum filter volume), followed by centrifugation at 14,000 × *g* for 20 min. The filtrate was carefully removed by pipetting and discarded. Molecular grade $H_2O$ was added back to the filter (up to a total volume of 500 μl), and the columns were centrifuged at 14,000 × *g* for 30 min (or until the volume was reduced to 25 μl). The Amicon® filters then were inverted in new sterile tubes and centrifuged at 1000 × *g* to recover the concentrated DOP-PCR product.

### 2.8. Multiplex STR amplification

Amplification of 16 STR loci was carried out using the AmpF*l*STR® Identifiler® Plus PCR Amplification Kit (Life Technologies, Foster City, CA). 10 μl of the concentrated DOP-PCR product were combined with 15 μl of master mix (for a total reaction volume of 25 μl). The master mix (per sample) consisted of 10 μl of AmpF*l*STR® Identifiler® Plus Master Mix and 5 μl of AmpF*l*STR® Identifiler® Plus Primer Set. Negative and positive controls were 10 μl of $TE^{-4}$ buffer and 10 μl 9947A Control DNA (0.1 ng/μl), respectively. PCR amplification was performed on the ABI GeneAmp® 9700 PCR System (Life Technologies, Foster City, CA) for 28 cycles, following the manufacturer's recommendations.

### 2.9. Post-PCR purification and CE analysis

All samples (both non-WGA-amplified extracts and DOP-PCR products) were purified using the Qiagen MinElute® Post-PCR Purification Kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. Purified Identifiler® Plus-amplified products were size-separated and detected on an ABI 3500*xl* Genetic Analyzer (Life Technologies) using 1 μl PCR product, 8.7 μl of Hi-Di™ Formamide, and 0.3 μl of GeneScan™ 600 LIZ® Internal Lane Size Standard. 1 μl of AmpF*l*STR Identifiler® Plus allelic ladder was included at least once per injection on the 96-well plate. All samples were denatured at 95 °C for 5 min and then immediately cooled on ice for 5 min. Electrophoresis was performed on a 36-cm capillary array with POP-4™ polymer (Life Technologies) using standard (default) injection time (10 s). The collected STR data were sized and typed with GeneMapper® ID-X Software Version 1.2 (Life Technologies) using a 75 RFU analytical threshold.

## 3. Results and discussion

The modified DOP-PCR primers were hypothesized to improve typing success of degraded DNA. The original primer (and 10N dcDOP primer) contained a restriction site because cloning of fragments was desired in the initial study. The restriction site in itself was never intended to contribute to amplification success and can be removed. Thus, there was more flexibility in primer design. The original primer design (i.e. six defined bases on the 3′ end of the primer) will identify on average a site in the genome approximately every 4000 bases. Therefore, the original primer should be more effective for relatively intact DNA than for degraded samples, as such long fragments may not be available for DOP-PCR. Two of the four newly-modified primers were designed so that the 3′ end would sit on average approximately every 256 bases to potentially amplify shorter fragments.

### 3.1. Modified DOP-PCR with high quality (non-degraded) DNA

Amplification of high-quality cell-line DNA with each of the seven degenerate primers was performed to determine if the reactions were working and to assess which primer(s) performed better. Preliminary investigations during this study demonstrated that DOP primer assays outperformed standard STR typing and the six modified DOP primers outperformed the originally reported DOP primer [2] in terms of increased RFU levels and recovery of alleles. Based on these results, the study proceeded with focus on the modified primers. Two different input template amounts (100 pg and 500 pg) of female 9947A and male 007 control DNA were used for proof-of-concept prior to using the primers on damaged and LCN samples. All six primers improved STR profiling performance using both 9947A and 007 templates (Supplementary Tables 1–4).

In general, with high-quality DNA the data indicated that all primers increased STR allele signals. However, consistent with previous studies [17–18], a significant number of artifacts were observed when high-quality, non-degraded DNA is used as DOP-PCR template. For the 100 pg 9947A comparison, the total average RFU was higher for the 10N dcDOP primer, skewed by the vWA locus. The 12N(2) abDOP primer outperformed the 10N dcDOP primer at nine loci (D7S820, D3S1358, THO1, D21S11, D19S433, TPOX, amelogenin, D18S51, FGA) and performed comparably at D8S1179, CSF1PO, and D16S539. For the 500 pg 9947A control DNA comparison, both the 12N and 12N(2) abDOP primers outperformed the 10N dcDOP primer in terms of total average RFUs. For the 100 pg 007 control DNA comparison, the 12N abDOP primer performed best, and 12N(2) abDOP, 14N abDOP, 10N dcDOP, and

16N dcDOP performed comparably. Lastly, for the 500 pg 007 comparison, the 12N and 12N(2) abDOP primers performed best in terms of average RFUs.

Given the overall data with high-quality DNA, the modified 12N abDOP, 12N(2) abDOP, and 10N dcDOP primers were selected for further evaluation with degraded and LCN templates. Only three primers were assessed further due to limited quantity/volume of extract available for testing. Table 2 summarizes all results with these modified primers for each of the four sample types used in this study: contemporary human skeletal remains, American Civil War era skeletal remains, World War II skeletal remains, and bloodstains.

### 3.2. Modified DOP-PCR with human skeletal remains

#### 3.2.1. Contemporary human skeletal remains

DOP-PCRs were carried out on DNA from contemporary human skeletal remains. Supplementary Table 5 shows an example of DOP-PCR results with degraded DNA from a contemporary human bone, comparing the maximum input into the standard amplification but allowing for greater amount of input (i.e., 1 ng) into the WGA reaction. Often with limited DNA samples, concentrations are low. Thus, a maximum input is limited to the 10 µl volume of sample that can be added to the PCR. Larger volumes (i.e., up to 50 µl) can be added to the WGA reaction, permitting flexibility of sample quantity for analysis and potentially reducing some of the stochastic effects that may occur. With this particular sample, 413 pg of initial input DNA yielded a very low RFU profile when amplified with the Identifiler® Plus PCR amplification kit (Supplementary Fig. 1A). However, with DOP-PCR, more input DNA was placed in the DOP-PCR than the standard STR PCR. The yields (in terms of RFUs) were much greater than the difference in input amounts for the before DOP-PCR analysis and the after DOP-PCR analysis. With this sample, the degree of allele dropout was comparable among the DOP-PCR primers used. However, both the 12N abDOP and 12N(2) abDOP primers outperformed the 10N dcDOP primer (in terms of increased RFU peak heights) at the majority of loci examined. Very few artifacts appeared in any of the resulting electropherograms (Supplementary Fig. 1B–D).

#### 3.2.2. Historical (American Civil War era) human skeletal remains

A set of historical skeletal remains (120-year-old American Civil War bones) also were subjected to WGA with the three modified DOP-PCR primers. It should be noted that no single extract from these remains yielded a full STR profile when initially examined (i.e. prior to WGA). Fifty different bone sections (tibiae, femora, and teeth) were extracted via three different methods, amplified with reagents from the AmpFlSTR Identifiler® Plus PCR amplification kit, and the results were compiled to generate a consensus profile [19]. Supplementary Table 6 and Fig. 2A show DOP-PCR results using 489 pg of non-WGA input template DNA from one example of a femur section of these remains. A 489 pg DNA sample generally should yield a complete STR profile, unless the DNA was compromised (as was observed with this sample). The same amount of DNA (489 pg) was used for DOP-PCR in this example to show the effects with the same amount of input DNA. The RFU values at all common loci that yielded results were higher than for the no DOP-PCR sample. In addition, several alleles that had previously dropped out were recovered. Also noteworthy, the majority of the alleles that were recovered as a result of DOP-PCR were consistent with the alleles in the compiled consensus profile previously reported [19] (Supplementary Table 6 and Fig. 2B–D). With this sample, the 10N dcDOP and 12N abDOP primers provided the highest STR signals.

Supplementary Table 7 depicts DOP-PCR results of another sample (tibia) from the 120-year-old historical remains using the same 519 pg input DNA as template for standard STR typing and for the DOP-PCR. As with the femur sample described in Supplementary Table 6, the RFU values at most loci increased and were similar among the DOP-PCR primers. However, 12N abDOP and 12N(2) abDOP-PCR primers gained a total of three additional alleles than had been observed by standard STR typing. All alleles observed and recovered as a result of the DOP-PCR were consistent with the previously compiled consensus profile [19] (electropherograms not shown).

DOP-PCRs with each of the three modified degenerate primers were carried out on eight additional femur samples, six additional tibia samples, and two teeth (for a total of 48 additional DOP-PCRs). Each reaction (using the three modified degenerate primers) resulted in improved STR profiles compared with pre-WGA typing (data not shown). Supplementary Table 8 compares the total average signal (RFU) per locus for all 48 DOP-PCRs for the same sample set with each of the modified primers. As shown in this table, the 12N abDOP and 12N(2) abDOP primers outperformed the 10N dcDOP primer at the majority of loci. Although the differences in peak heights between the primers were not substantial, there was a trend supporting small improvements which can be important with LCN and degraded samples. It is possible, though, that these differences are due to stochastic sampling effects rather than a difference in performance between the primers. Also of note is that the two alleles at the D2S1338 locus (which were not observed at all in the original consensus profile) were recovered in fourteen of the 48 subsequent DOP-PCRs (7 from tibia samples, 7 from femur samples). More importantly, these recovered D2S1338 alleles (i.e. alleles 17 and 19) were consistent among the fourteen WGA reactions and for all three modified degenerate primers.

This larger sampling is consistent with this study's hypothesis that re-design of the degenerate primer would increase the number of potential annealing sites (and therefore would be more effective at amplifying the shorter fragments of DNA prevalent in degraded samples). However, the data suggest that any of the three modified primers could serve as degenerate primers for screening a compromised sample.

Although the consensus testing method involving multiple DNA extractions (such as with the fifty DNA extractions that were performed on this set of American Civil War era skeletal remains) is a common approach for improving the reliability of STR typing of ancient and forensic bone samples [21,22], there may be instances

**Table 2**
Summary of DOP-PCR results with three different modified primers for four different sample types: contemporary human skeletal remains ($n = 5$), American Civil War era skeletal remains ($n = 50$), World War II skeletal remains ($n = 25$), and environmentally damaged bloodstains ($n = 5$).

| Sample type | Modified primer | Average RFU | Average # of alleles |
|---|---|---|---|
| Contemporary bones | None | 130 | 20 |
| Contemporary bones | 10N dcDOP | 899 | 28 |
| Contemporary bones | 12N abDOP | 1229 | 28 |
| Contemporary bones | 12N(2) abDOP | 1332 | 28 |
| American Civil War era bones | None | 266 | 17 |
| American Civil War era bones | 10N dcDOP | 681 | 22 |
| American Civil War era bones | 12N abDOP | 829 | 22 |
| American Civil War era bones | 12N(2) abDOP | 934 | 23 |
| World War II bones | None | 351 | 17 |
| World War II bones | 10N dcDOP | 2286 | 25 |
| World War II bones | 12N abDOP | 1070 | 22 |
| World War II bones | 12N(2) abDOP | 1929 | 26 |
| Human bloodstains | None | 529 | 19 |
| Human bloodstains | 10N dcDOP | 1745 | 21 |
| Human bloodstains | 12N(2) abDOP | 2707 | 23 |

in which this method is not feasible (either due to lack of sufficient bone material for testing, or in scenarios involving museum or archaeological specimens where the physical and structural integrity of the bone must be preserved).

*3.2.3. World War II human skeletal remains*

All DNA extracts from unidentified Finnish World War II soldiers yielded partial STR profiles in pre-WGA.

Supplementary Table 9 shows results of DNA from one of the WWII samples that was amplified with all three modified degenerate primers. Since the maximum volume of extract that can be added to the Identifiler® Plus PCR amplification reaction was 10 μl, the "before DOP-PCR" quantity listed in the table (664 pg) represents the maximum amount of DNA used in pre-WGA genotyping. Given that initial STR typing yielded a partial profile with low RFU levels, a greater amount of DNA (i.e., 996 pg) was used for the subsequent DOP-PCRs. With this sample, the 10N dcDOP primer yielded the highest average signal of STR products. Allele recovery was greater with WGA for the three modified DOP-PCR primers compared with standard STR typing.

DOP-PCRs with each of the three modified degenerate primers were carried out on four of these WWII skeletal remains (for a total of 24 additional DOP-PCRs). All reactions (using the three modified degenerate primers) resulted in improved STR profiles compared with pre-WGA typing. Supplementary Table 10 compares the total average signal (RFU) per locus for all 24 DOP-PCRs for the same sample set with each of the modified primers. A summary table (Table 2) shows the total average signal (RFU) across all loci and the total average number of alleles after DOP-PCR. With this set of samples, the 10N dcDOP primer performed higher on average in terms of total signal (RFU) obtained per locus, but the 12N(2) abDOP primer performed best in terms of total number of alleles recovered.

*3.3. Modified DOP-PCR with degraded DNA from environmentally-damaged human bloodstains*

Because the recovery of DNA from the bloodstains was low, only two modified primers were tested. DOP-PCR using the 10N dcDOP and 12N(2) abDOP primers was performed on damaged DNA from a human bloodstain that had been environmentally-insulted for 24 weeks. Selection of the 12N(2) abDOP primer over the 12N abDOP primer was supported based on the results from the bone studies. The amount of input template was varied to assess the range of input DNA needed to obtain optimal results.

Both the 10N dcDOP and 12N(2) abDOP primers were effective at improving STR profiling of the sample, with the latter primer yielding the higher signal at all three input levels (Supplementary Table 11).

Although with this sample the pre-DOP-PCR yielded a full STR profile with 657 pg DNA, this quantity of input DNA was amplified to screen for potential artifacts that may be peculiar to the WGA reaction. The resultant electopherograms (Supplementary Figs. 3A–C and 4A–C) showed that in our hands the assertion that addition of more than 100 pg of DNA resulted in significant artifacts (making results uninterpretable) does not necessarily apply when the candidate template is substantially degraded.

Supplementary Table 12 shows results of another environmentally-damaged bloodstain that was amplified with the 10N dcDOP primer and 12N(2) abDOP primer. The "before DOP-PCR" quantity (728 pg) represented the maximum permitted amount of DNA used in pre-DOP-PCR typing. Given that initial STR typing yielded a partial profile with low RFU levels (Supplementary Fig. 5A) and a greater volume could be added to the WGA reaction, 1 ng of input template DNA was used for the subsequent DOP-PCRs (Supplementary Fig. 5B and C). Similar to the previous example, both primers performed better than a no-DOP-PCR process.

Another environmentally-damaged bloodstain yielded a partial, low RFU STR profile with non-WGA treated DNA (Supplementary Table 13 and Fig. 6A). Again, since the maximum volume of extract that can be added to the Identifiler® Plus PCR amplification reaction was 10 μl, the "before DOP-PCR" quantity (107 pg) represented the amount of DNA used in pre-DOP-PCR typing. Also, 1 ng of input template DNA was used for the DOP-PCR. Results using the 10N dcDOP and 12N(2) abDOP primers are shown in Supplementary Fig. 6B and C.

DOP-PCR results with this limited data set of environmentally-insulted bloodstains indicate that the 12N(2) abDOP primer generally works better in terms of increasing allele peak heights and recovery of alleles but the 10N dcDOP primer worked as well. Furthermore, the results showed that addition of more than 100 pg of template to the DOP-PCR in general does not produce excessive artifacts to the degree that the profile may become difficult to interpret.

With any assay that increases sensitivity of detection there is the potential of allele drop-out, allele drop-in, and increased stutter. These same artifacts were observed in the STR profiles generated after DOP-PCR. Examples of such artifacts are labeled in Supplementary Figs. 1–6. Overall, these artifacts were not excessive. The impact and interpretation on the bone samples, which are single source samples, often is not as great as it might be for more complex samples such as mixtures. There were a number of examples that could illustrate this point but only a couple of examples are provided. In Supplementary Fig. 2C, three alleles were detected at the D3S1358 locus (alleles 15, 17, 18). Given the overall peak heights, the evidence would strongly favor that the 17 and 18 alleles are the true alleles and the 15 allele is drop-in. The consensus profile by standard STR typing of this sample confirmed that 17 and 18 are the true alleles. In Supplementary Fig. 6C at the vWA locus there are three alleles detected (alleles 16, 19, 20). Given the overall peak heights, the 16 and 19 alleles are more likely the true alleles and allele 20 is likely increased stutter (or potential allele drop-in). Indeed, 16 and 19 are the true alleles of the sample donor. These examples show that interpretations with such artifacts may not be as difficult as what may be encountered with more complex profiles.

Lastly, attention is directed to Supplementary Fig. 4B. There are a number of extra peaks per locus, which is indicative of contamination. This observation is particularly noteworthy as the DNA for profiles displayed in Supplementary Fig. 4A and C did not display evidence of such contamination and the DNA for all three profiles came from the same extract. Moreover, all negative controls were blank. The contamination in 4B (after subtracting alleles common in 4A) did not match any alleles of anyone who was involved in the handling of these samples. The likely explanation is contamination that was specific to the tube or pipette tip used to handle the DNA aliquot of the 4B sample. The result in 4B emphasizes the limitations of negative controls in monitoring potential contamination.

## 4. Conclusion

This study was successful in using a modified DOP-PCR to improve STR profiling of damaged and LCN DNA samples. The modifications to the primers [particularly the 12N(2) abDOP primer] allowed for better typing results for a portion of the environmentally-damaged bloodstains, contemporary human skeletal remains, American Civil War era bone samples, and skeletal remains of WWII soldiers over that obtained by previously-described DOP-PCR methods and their primers. In some cases, the 10N dcDOP primer performed better than the newly modified primers described in this study. However, in all cases the DOP-PCR

performed better than routine STR typing. As with any samples with low amounts of template DNA that were subjected to increased sensitivity of detection analyses, some exaggerated stochastic effects were observed. These properties are inherent in LCN typing assays and are not novel observations [23–26]. More importantly, no new types of artifacts were observed. While such effects impact the ability to interpret results and apply statistical assessments, the stochastic artifacts and contamination of DOP-PCR-treated samples were nominal and consistent with results from other LCN typing practices.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.legalmed.2015.10.013.

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