Legal Medicine 18 (2016) 7–12

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

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

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ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form 28 August 2015
Accepted 30 October 2015
Available online 11 November 2015

Keywords:
DOP-PCR
Whole genome amplification
Degraded DNA
Human skeletal remains
Bloodstains

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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http://dx.doi.org/10.1016/j.legalmed.2015.10.013
1344-6223/Published by Elsevier Ireland Ltd.

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