Forensic Science International: Genetics 32 (2018) 40–49

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Review article

Direct PCR amplification of forensic touch and other challenging DNA samples: A review

Sarah E. Cavanaugh*, Abigail S. Bathrick

Bode Cellmark Forensics, Inc., 10430 Furnace Road, Suite 107, Lorton, VA 22079, USA

ARTICLE INFO

Keywords:
Forensic identification
DNA typing
Direct PCR
Biological evidence
STR profile
Touch DNA

ABSTRACT

DNA evidence sample processing typically involves DNA extraction, quantification, and STR amplification; however, DNA loss can occur at both the DNA extraction and quantification steps, which is not ideal for forensic evidence containing low levels of DNA. Direct PCR amplification of forensic unknown samples has been suggested as a means to circumvent extraction and quantification, thereby retaining the DNA typically lost during those procedures. Direct PCR amplification is a method in which a sample is added directly to an amplification reaction without being subjected to prior DNA extraction, purification, or quantification. It allows for maximum quantities of DNA to be targeted, minimizes opportunities for error and contamination, and reduces the time and monetary resources required to process samples, although data analysis may take longer as the increased DNA detection sensitivity of direct PCR may lead to more instances of complex mixtures. ISO 17025 accredited laboratories have successfully implemented direct PCR for limited purposes (e.g., high-throughput databanking analysis), and recent studies indicate that direct PCR can be an effective method for processing low-yield evidence samples. Despite its benefits, direct PCR has yet to be widely implemented across laboratories for the processing of evidentiary items. While forensic DNA laboratories are always interested in new methods that will maximize the quantity and quality of genetic information obtained from evidentiary items, there is often a lag between the advent of useful methodologies and their integration into laboratories. Delayed implementation of direct PCR of evidentiary items can be attributed to a variety of factors, including regulatory guidelines that prevent laboratories from omitting the quantification step when processing forensic unknown samples, as is the case in the United States, and, more broadly, a reluctance to validate a technique that is not widely used for evidence samples. The advantages of direct PCR of forensic evidentiary samples justify a re-examination of the factors that have delayed widespread implementation of this method and of the evidence supporting its use. In this review, the current and potential future uses of direct PCR in forensic DNA laboratories are summarized.

1. Introduction

Improved methods to generate high-quality DNA profiles from samples that yield low amounts of DNA are of considerable interest to forensic DNA laboratories. Direct polymerase chain reaction (PCR) amplification, a sample processing method in which an evidence swab or substrate punch is added directly to an amplification reaction without prior extraction or quantification, may improve the generation of genotyping data from such samples. However, most laboratories continue to use standard methods to process low-level sample types. In part, this is due to guidelines issued by forensic DNA expert groups and advisory boards that prevent many laboratories from implementing direct PCR amplification of forensic evidence samples. In the United States, for example, the Federal Bureau of Investigation (FBI) Quality Assurance Standard (QAS) 9.4 requires all unknown forensic samples to undergo human-specific DNA quantification prior to amplification of short tandem repeat (STR) loci [1]. The Organization of Scientific Area Committee (OSAC) for Forensic Science has identified the need to re-evaluate such guidelines, specifically in relation to touch DNA evidence samples.

Touch DNA evidence samples are frequently collected from items on which skin cells have been deposited after being worn or handled. Various factors can affect the amount of DNA transferred to an object, including donor perspiration, the donor's propensity to shed epithelial cells, frequency of hand washing, tendency of the donor to touch other areas of the body, pressure and friction during contact, and type of surface being handled [2–7]. However, it is generally understood that touch DNA samples typically contain low amounts of DNA [4]. Forensic interest in touch DNA has been steadily increasing since the early 2000s, particularly in relation to property crime investigations [8–10].

* Corresponding author.
E-mail addresses: sarah.cavanaugh@bodetech.com (S.E. Cavanaugh), abby.bathrick@bodetech.com (A.S. Bathrick).

http://dx.doi.org/10.1016/j.fsigen.2017.10.005
Received 14 July 2017; Received in revised form 11 October 2017; Accepted 16 October 2017
Available online 18 October 2017
1872-4973/

As literature regarding the successful use of touch DNA samples in property crime investigations becomes more prolific, many crime laboratories can expect increases in the number of touch DNA evidence samples that are submitted for DNA analysis, which can lead to increased turnaround times, backlogs, and processing costs. Additionally, inaccurate sample targeting and poor collection techniques can result in submission of samples that do not generate meaningful results, leading to a loss of resources and reduced confidence in the process [11]. In response to this, several studies have evaluated the success rates of touch DNA evidence samples [12–15]. In general, these studies have demonstrated that care must be taken when deciding what types of touch DNA evidence items to submit for DNA processing, as some item types are more likely to produce complex mixtures or no results [12]. Such studies can be used to aid in the establishment of sample collection and submission guidelines that improve the success rates associated with touch DNA evidence items.

The success of touch DNA samples is also affected by the laboratory methods with which they are processed. Standard workflows for processing touch DNA evidence samples involve DNA extraction, quantification, and STR amplification. DNA extraction and quantification both result in the loss of a portion of the original sample and increase the opportunities for exogenous DNA to be introduced to the sample. DNA extraction purifies nucleic acid from cellular debris, endogenous proteins, and exogenous inhibitors that may interfere with enzyme activity during PCR amplification of STR loci. However, DNA extraction can result in an approximate loss of 20% to 90% of the initial template amount, dependent upon the method of extraction and accuracy of the quantification method [16–18]. Factors that affect DNA extraction efficiency include the number of tube changes, the number of washing steps, and the capacity of DNA to absorb/irreversibly bind to plastic consumables [19] and extraction matrices [20]. For example, in silica-based extraction protocols, the silica matrices contain a small percentage of irreversible binding sites that permanently bind nucleic acid [21] and may contribute to sample loss if carrier RNA is not present in the extraction [22]. The necessity of DNA extraction for touch DNA samples has been questioned [23,24] due to the presence of cell-free DNA. Cell-free DNA in touch samples is believed to result from apoptosis of epithelial cells. It is often lost during extraction and has been observed in the supernatant of 90% of the biological samples evaluated in a recent study [23]. Quinones and Daniel concluded that, to maximize profiling success of touch DNA, the cell-free DNA component of touched surfaces should be retained and included in sample processing [25]. By eliminating the DNA extraction step, cell-free DNA remains available as an additional source of DNA during direct PCR and may contribute to improved profile generation.

In standard DNA processing workflows, DNA quantification is seen as critical to assess the amount of human DNA extracted, select the optimal amount of DNA extract to amplify, and determine the most appropriate downstream methods of genotyping analysis [26]. In addition, quantification can be used to determine the amount of male DNA present in sexual assault samples and can reveal the presence of degraded DNA and PCR inhibitors [26]. Estimation of DNA concentration is particularly valuable as STR amplification kits are designed to work with specific DNA template ranges for optimal profile generation and minimization of stochastic effects [26]. While the extreme sensitivity of commercially available quantitative PCR (qPCR) assays may allow for the detection of samples containing DNA quantities below STR amplification sensitivity, attempts to establish a definitive quantification value threshold below which no STR profile can be detected have not been successful [27–29]. Sampling inaccuracies and stochastic fluctuations commonly encountered with low-level DNA samples prevent qPCR from functioning as an infallible sample screening technique [11]. Moreover, during quantification, typically 2 µl of DNA extract is removed from the final elution volume, which further reduces the total amount of DNA available for STR or amplification. Following quantification, concentration of low-yield samples may be required to maximize the amount of template added into amplification reactions. An evaluation of commonly used centrifugal filter devices showed DNA loss between 33% and 67% after concentration [30]. DNA loss during centrifugal concentration has been attributed to entrapment of DNA in the device, which can be minimized through membrane pre-treatment with RNA [31].

The use of direct PCR amplification has been suggested to combat DNA loss from touch evidence samples [11,24]. By circumventing the extraction, quantification, and concentration processes, maximum quantities of DNA can be targeted, laboratory personnel error and exogenous DNA contamination may be minimized, and overall sample processing time and cost could be reduced. Given that many conventional amplification systems require more than 100 pg to consistently generate full profiles [32–35], a sample would initially need to contain approximately 250 cells (1.45 ng) to retain a sufficient amount of DNA for amplification after extraction, whereas a direct PCR workflow only requires approximately 17 cells [17], without taking into account cell-free DNA. Direct PCR amplification has been in use in molecular biology since the 1990s, first for colony PCR, a rapid screening method for large numbers of bacterial cells for a gene of interest [36], then for human leukocyte antigen (HLA) testing of whole blood [37]. Direct PCR is now routinely used for viral and bacterial pathogen detection in clinical specimens [38,39] as well as DNA barcoding of taxa for biodiversity monitoring and species identification [40,41].

2. Direct PCR of reference samples

Use of direct PCR on forensic reference samples began in the mid–2000 s with the release of commercial direct STR amplification kits, which use advanced buffer-polymerase technology that can overcome the influences of many common PCR inhibitors [42]. Thermo Fisher Scientific offers direct PCR versions of their Applied Biosystems™ autosomal STR and Y-STR amplification kits: AmpFlSTR® Identifiler® Direct, AmpFlSTR NGM SElect™ Express, GlobalFiler® Express, and AmpFlSTR Yfiler® Direct. Promega offers PowerPlex® 18D specifically for direct PCR; however, the remainder of their current product line (PowerPlex Y23, PowerPlex 21, PowerPlex Fusion, and PowerPlex Fusion 6C) all have proven direct PCR protocols, allowing the same kit to be applied to casework and reference database samples. Additionally, QIAGEN® offers the Investigator® IDplex GO! and Investigator 24plex GO! Kits. Primer sequences contained in direct PCR kits are identical to their standard amplification kit counterparts. Table 1 provides a comparison of the current commercially available direct PCR kits for the analysis of autosomal STRs. Depending on the kit manufacturer, addition of a pre-treatment wash or lysis buffer may be recommended (Table 1). Pre-treatment buffers initiate cell lysis directly on a 1.2 mm punch of the sample/collection matrix within a PCR reaction well and are either evaporated prior to the addition of PCR reaction mix or remain in the well during PCR; no DNA purification is performed. When a whole swab head is utilized, lysis is performed in a larger volume. The swab head is then removed, and an aliquot of the lysate is added to the direct PCR reagents. According to the manufacturers, modifications to the direct PCR amplification buffers allow for more balanced amplification in the presence of possible inhibitors and higher concentrations of DNA [53]. Commercial buffer systems are comprised of proprietary recipes containing optimized concentrations of magnesium salt, primer pairs, nucleotides, bovine serum albumin (BSA), proprietary hot-start thermostable polymerases, and other additives that help mitigate the effects of contaminating inhibitory chemicals [33,34,51]. These systems are also tolerant of larger DNA input quantity ranges, often upwards of 5–10 ng [53,54]. Target sample types for use with these kits are reference blood or buccal cells on treated (i.e., Whatman FTA® or Copan NUCLEIC-CARD™) and untreated (i.e., Bode Buccal DNA Collector™ or 903 paper) paper-based substrates and sample swabs.

Reference samples typically contain high quantities of relatively high quality DNA in a standardized format and are amenable to direct