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Research paper

Development of a normalized extraction to further aid in fast, high-throughput processing of forensic DNA reference samples

Catherine C. Connon[a,b,c,*], Aaron K. LeFebvre[b,d], Robert C. Benjamin[c]

[a] Virginia Commonwealth University, Department of Forensic Science, 1015 Floyd Ave Box 843079, Richmond, VA 23284, United States
[b] Cellmark Forensics, Inc., a LabCorp Specialty Testing Group, 13988 Diplomat Drive, Suite 100, Farmers Branch, TX 75234, United States
[c] University of North Texas, Department of Biological Sciences, 1155 Union Circle #305220, Denton, TX 76203-5017, United States
[d] Next Health LLC, 5710 Lyndon B Johnson Fwy #300, Dallas, TX 75240, United States

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ABSTRACT

The goal of this project was to develop a "normalized" extraction procedure to be used in conjunction with previously validated 3 μL fast PCR reactions (42–51 min utilizing KAPA2G™ Fast Multiplex PCR Kit) and alternative capillary electrophoresis (24–28 min injection using POP-6™ Polymer and a 22 cm array). This was the final phase of a workflow overhaul for the database unit at Cellmark Forensics to achieve a substantial reduction in processing time for forensic DNA database samples without incurring significant added costs and/or the need for new instrumentation, while still generating high quality STR profiles. Extraction normalization aimed to consistently yield a small range of DNA concentrations, thereby eliminating the need for sample quantification and dilution. This was specifically achieved using the ChargeSwitch® Forensic DNA Purification Kit and a reduction in extraction bead quantity, thereby forcing an increase in bead binding efficiency. Following development of this extraction procedure, an evaluation ensued to assess the combination of normalized extraction, 3 μL fast PCR (with PowerPlex 16 HS, Identifiler Plus and Identifiler primer sets), and alternative CE detection – further referred to as new "first pass" procedures. These modifications resulted in a 37% reduction in processing time and were evaluated via an in depth validation, from which nearly 2000 STR profiles were generated, of which 554 profiles from 77 swab donors and 210 profiles from 35 buccal collector donors specifically arose from the new first pass procedures. This validation demonstrates the robustness of these processes for buccal swabs and Buccal DNA Collectors™ using the three primer sets evaluated and their ability to generate high quality STR profiles with 95–99% and 88–91% pass rates, respectively.

1. Introduction

As technology continues to advance, there is a certain expectation that such advancements will result in a decrease in processing time and cost. Previous studies have demonstrated the ability to reduce processing time via fast PCR and/or direct amplification [1–12], while capillary electrophoresis detection time can be reduced using a combination of POP-6™ Polymer (POP-6; Applied Biosystems, Foster City, CA) and a 22 cm array [13] or using a 3500 series Genetic Analyzer (Applied Biosystems) [14]. Rapid DNA testing has also been achieved in less than two hours from start to finish using a single instrument, and has demonstrated its suitability for investigative leads when time is a crucial factor [15,16], though it is not a cost effective means of testing for high-throughput laboratories. For high-throughput laboratories, time and cost effectiveness are critical, and often times, the means to achieving a reduction in processing time include added costs (e.g., use of fast thermal cyclers and fast polymerases).

Cellmark Forensics underwent an ongoing long-term project to develop a new "first pass" process for DNA reference samples with several very specific goals. The new process must be high-throughput, robust enough for a variety of reference sample types and forensic STR primer sets, substantially reduce processing time (including elimination of quantification and dilution step prior to amplification), not require any new instrumentation, not require a significant increase in reagent costs, utilize a low volume (preferably 3 μL) amplification to keep costs down, and most importantly, must continue to result in high quality STR profiles with about a 90% (or higher) first pass success rate. Initial progress was made via the development of an alternative capillary electrophoresis (CE) detection method utilizing POP-6 polymer and a 22 cm array on a 3130*xl* Genetic Analyzer (3130xl; Applied

* Corresponding author at: Virginia Commonwealth University, Department of Forensic Science, 1015 Floyd Ave Box 843079, Richmond, VA 23284, United States.
E-mail address: cmconnon@vcu.edu (C.C. Connon).

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Biosystems), as well as low volume (3 μL) fast PCR amplifications [1,2,13].

This paper focuses on the final phase of this project – the elimination of quantification and dilution of DNA samples prior to amplification, and integration of all three modules to produce a smooth first pass procedure. Despite the fact that direct amplification would save a substantial amount of time via the need for little if any sample preparation, as well as the elimination of quantification and dilution, it has resulted in some negative side effects with respect to STR profile quality, including PCR inhibition and incomplete adenylation [8,11]. Furthermore, it would prevent the use of a 3 μL PCR reaction due to the presence of a physical substrate; such a substantial reduction in reaction volume would lead to a tremendous cost savings (e.g., a 25 μL Identifiler Plus reaction is roughly $16-$17/sample, whereas a 3 μL reaction is about $2). Alternatively, Cellmark Forensics chose to develop a "normalized" extraction, one that would consistently result in a narrow window of DNA concentrations that was suitable for amplification immediately following extraction, and therefore able to bypass the need for quantification and dilution. Given the cost savings associated with the 3 μL reaction, coupling an extraction step (which costs about $0.60/sample in Cellmark's databasing unit) with the low volume amplification, offers a sizeable costs advantage over a direct amplification alone (close to the cost of the full volume amplification reaction).

In order to achieve such an endeavor, it was theorized that a bead-based extraction could be utilized in which a small volume of beads was used per sample in an attempt to force the beads to reach maximum DNA binding capacity. The ChargeSwitch® Forensic DNA Purification Kit (ChargeSwitch; Invitrogen, Carlsbad, CA) was pursued because it had been the primary method of extraction for Cellmark Forensics' databasing unit for a number of years, primarily because it helped maintain a high (>90%) "first pass" success rate, was amenable to automation on a 96-well platform, was a relatively quick process (1–2h incubation and ~25 min purification process), and was less expensive than other bead-based extractions. Since DNA yields can vary from individual to individual, it was important to make sure enough DNA (i.e., sample cutting size) was extracted so that even the lowest material would supply enough DNA to saturate the beads, or at least provide enough to be within the optimal DNA input range (0.375–1.5 ng) for the low volume fast PCR protocols previously developed for this project [2].

The development of a normalized extraction and validation of the new "first pass" process for reference DNA samples – specifically, buccal swabs and Buccal DNA Collectors™ (buccal collectors; Bode Cellmark Forensics, Lorton, VA) – are presented here.

2. Materials and methods

2.1. General overview

2.1.1. Sample preparation and extraction

For all studies, buccal swabs and buccal collectors were stored at room temperature in a climate controlled area, away from sunlight. Buccal swab cuttings (~1/4 swab) or Buccal DNA Collector™ punches (6 mm) were extracted (quarter reaction with a 1–2 h incubation at 56 °C) using the ChargeSwitch kit [17] in a 96-well plate on a BioSprint 96 (QIAGEN, Hilden, Germany) or KingFisher® 96 (Thermo Fisher Scientific, Waltham, MA). All reagents were included in the ChargeSwitch kit and were prepared as directed, except that the supplied Lysis Buffer was diluted 50% with sterile water. Per the laboratory's current, validated, non-normalized extraction procedure, 300 μL of 50% Lysis Buffer and 5 μL of Proteinase K were added to the swab/punch substrates in a deep-well 96-well plate. The plate was sealed and vortexed for 5 s, followed by a 2 min sonication. Next, samples were incubated at 56 °C for a minimum of 1hr but no more than 2hr (i.e., 1–2 h) for buccal swabs and overnight for punches. Following incubation, all lysate was transferred to a clean 96-well plate to separate it from the substrate. Next, 100 μL of Purification Buffer and 5 μL of ChargeSwitch extraction beads were added to each sample, and the 96-well plate was loaded on a BioSprint 96 or KingFisher® 96 for DNA purification. Samples were washed with 125 μL of Wash Buffer 1, followed by the same volume of Wash Buffer 2. Lastly, buccal swabs were eluted in 80 μL of Elution Buffer, while buccal collectors were eluted in 60 μL.

Several alternate extraction parameters that strayed from the manufacturer's protocol were assessed during the developmental stages of a normalized extraction. Volumes of Proteinase K (5.0 μL or 10 μL), beads (0.25–5.0 μL), Wash Buffer 1 and 2 (125 μL each, 250 μL/125 μL or 250 μL each), and Elution Buffer (60 μL, 80 μL or 120 μL), as well as incubation length (1.5 h or overnight) and including or excluding a 5 s post-lysis vortex, were all tested with buccal swabs and/or punches. Buccal swab and collector samples were collected from individuals known to generally yield high, moderate, or low amounts of genetic material. For each parameter assessed, samples were processed in triplicate from four individuals or in duplicate from ten individuals. Once a set of potential normalized extraction parameters was identified, a larger test batch consisting of samples from 34 to 35 individuals (many of which had an unknown degrees of genetic material yields) was processed in duplicate.

Based upon information obtained from the normalized extraction developmental phase, no more than two cuttings or punches were ever collected from the same swab or punch, respectively, for the validation studies. The normalized extraction that was validated had the following modifications from the current, non-normalized procedure: 1) a reduction in bead volume from 5.0 μL to 0.5 μL for buccal swabs and 1.0 μL for buccal collectors and 2) a change in final elution volume to 60 μL for buccal swabs and 80 μL for buccal collectors. Together, these changes were designed so that the vast majority of extracted samples would fall within a targeted DNA concentration range of 0.42–1.67 ng/μL, which would coincide with the amplification of 0.375–1.50 ng DNA (the optimal DNA input range for 3 μL fast PCR, as established by Connon et al. [2]).

2.1.2. Quantification

DNA samples were quantified using the Quant-iT™ PicoGreen® dsDNA Quantitation Kit coupled with the Quant-iT™ PicoGreen® dsDNA Quantitation Reagent (PicoGreen; Invitrogen) and a FLUOstar microplate reader (BMG LABTECH, Ortenberg, Germany) [1,2] in order to assess DNA concentrations following extraction. This assisted in achieving the desired window of DNA concentrations (0.42–1.67 ng/μL). Though this is not a human specific means of quantification, Cellmark Forensics successfully utilized this procedure in their databasing unit from 2008 to 2015. Such a practice is in accordance with the FBI Quality Assurance Standards guidelines for reference samples in either casework or databasing laboratories because the system had been validated and proven to be reproducible and reliable [18,19].

At times, comparison to the current workflow was necessary, and this included a dilution step (using sterile water) following quantification in order to target 0.75–1.0 ng DNA for low volume, non-fast PCR amplification.

2.1.3. STR AMPLIFICATION

Samples were amplified via low volume (3 μL) fast PCR on a 384-well Veriti® thermal cycler (Veriti; Applied Biosystems) using