Forensic Science International: Genetics 22 (2016) 113–127

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Research paper

Validation of alternative capillary electrophoresis detection of STRs using POP-6 polymer and a 22 cm array on a 3130*xl* genetic analyzer

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., 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

ARTICLE INFO

Article history:
Received 3 October 2015
Received in revised form 6 February 2016
Accepted 10 February 2016
Available online 12 February 2016

Keywords:
Forensic science
Capillary electrophoresis
Alternative detection
Resolution
Precision

ABSTRACT

The goal of this project was to reduce capillary electrophoresis detection time on a 3130*xl* Genetic Analyzer for amplification product obtained from 4-dye and 5-dye STR amplification kits while still generating high quality STR profiles. This was accomplished by utilizing a more viscous polymer (POP-6™) and a shorter array (22 cm) than that which are typically used (POP-4® polymer and a 36 cm array) for human identification purposes. Spatial calibration and detection run modules were modified in response to the use of this polymer/array combination and to reduce detection time. Alternative detection resulted in 24–28 min run times, as compared to ~45 min using traditional POP-4®/36 cm detection methods. POP-6™/22 cm detection run modules were validated for use with 4-dye Promega STR kits (e.g., PowerPlex® 16 and PowerPlex® 16HS) and 5-dye Life Technologies kits (e.g., Identifiler® and Identifiler® Plus). Three hundred ninety-five samples, controls and allelic ladders were used for the validation studies, which consisted of a comparison of alternative POP-6™/22 cm detection to traditional POP-4®/36 cm (including reproducibility/concordance of allele calls, resolution, ILS sizing quality, peak height and pass rates), a sizing study (precision and accuracy) and a sensitivity study to obtain a usable range of injection times. Compared to traditional POP-4®/36 cm detection, alternative detection resulted in 100% reproducible and concordant alleles, the ability to achieve one base resolution, slightly reduced ILS sizing quality, slightly reduced peak height and statistically similar pass rates ($\alpha = 0.05$). It should be noted that alternative detection offered improved resolution over that of traditional for amplicons less than ~200 b, but had reduced resolution for products greater than ~200 b. Additionally, alternative detection yielded acceptable precision and accuracy of sizing using Life Technologies criteria (<0.15 standard deviation of allele sizing and ±0.5 b sizing differences for the same allele) and usable injection parameters of 2 kV 4–15 s (compared to 3 kV 10 s for traditional). The run modules developed and validated for 4-dye and 5-dye STR kits using POP-6™ polymer on a 22 cm array offer a tremendous reduction in detection time (~40%) while still generating high quality STR profiles.

1. Introduction

Capillary electrophoresis was first introduced in the late 1990s and has been used for the detection of short tandem repeats (STRs) for forensic purposes since the early 2000s [1–6]. Like traditional slab-gel electrophoresis, separation is based on the size and charge of each molecule, and can be controlled by the viscosity of the separation matrix, distance traveled through the matrix, time of travel, voltage and current [1,6,7]. Instead of using agarose or polyacrylamide as the separating matrix, the capillaries use a liquid polymer. Life Technologies manufactures three such polymers, Performance Optimized Polymer (POP-4® Polymer [POP-4], POP-6™ Polymer [POP-6] and POP-7™ Polymer [POP-7]; Life Technologies, Grand Island, NY) and four array lengths (22 cm, 36 cm, 50 cm and 80 cm) for use with their Genetic Analyzer instruments [8]. POP-4 and POP-6 are less viscous than POP-7, and each polymer is designed for a different purpose (Life Technologies, personal communications, November 11, 2014 and December 5, 2014; [9–11]). Life Technologies recommends using POP-4 for DNA sequencing and fragment analysis (i.e., microsatellites and SNPs) for fragments <500 b, and POP-4 is commonly used for human identification (HID; e.g., forensic applications)

* 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).

http://dx.doi.org/10.1016/j.fsigen.2016.02.006
1872-4973/

purposes. POP-6 is recommended for standard and rapid DNA sequencing, but is currently the only option for long sequence fragments (>500 b) on 310 and 3100 Genetic Analyzers. Like POP-4, POP-7 is recommended for DNA sequencing and fragment analysis, but offers the added capability of sequencing up to ~1000 b. However, it is not compatible with the 310 or 3100 instruments. The shortest array (22 cm) is used for fragment analysis and the longest (80 cm) is used for sequencing, whereas the 36 cm and 50 cm arrays can be used for either [8].

It appears that most laboratories in the forensic DNA community follow Life Technologies' recommendation to utilize POP-4 with a 36 cm array (on various models of Genetic Analyzers) for forensic STR/human identification (HID) applications, which takes about 45 min to process samples (on a per injection basis) on 3100/3130 series Genetic Analyzers [8,12,13,14,15], but some are also using or have used POP-6 with a 36 cm array [16].

Others using POP-6 appear to use a 50 cm array based upon a run time of 2.75 h, though the array length is not indicated [17]. POP-6 offers increased resolution as compared to POP-4 due to its higher viscosity. Although increased resolution may not necessary for HID purposes, it could be beneficial because *complete* separation [1] of alleles differing in size by one base cannot be achieved using the current HID conditions recommended by Life Technologies (POP-4 with a 36 cm array). Using POP-4 with a 36 cm array, the databasing facility at Cellmark Forensics had also observed – on the rare occasion – extremely poor resolution of larger amplicons differing by one base (e.g., alleles 13.4 and 14 at the Penta D locus of the PowerPlex® 16HS System [PowerPlex 16HS; Promega, Madison, WI], which are ~420 b), such that the analysis software could not differentiate between the two alleles (see Fig. 1).

To date, no published studies involving significant reductions in capillary electrophoresis run time have been identified, but preliminary unpublished work has previously been conducted in 2008 involving POP-6 on a 22 cm array at Cellmark Forensics that yielded results that looked promising for forensic STR usage. Furthermore, Azco Biotech manufactures polymer alternatives to Life Technologies' POP-4, POP-6 and POP-7, called NanoPOP4, NanoPOP6 and NanoPOP7, respectively (Azco Biotech, Inc., Oceanside, CA) [18]. The NanoPOP polymers include a higher percentage of a key proprietary ingredient found in Life Technologies' polymers, which results in faster run times than can be achieved using Life Technologies' polymer with as good or better resolution (A. Perlman, personal communication, January 26, 2013). However, Connon evaluated NanoPOP-4 on a 3130*xl* Genetic Analyzer (3130xl; Life Technologies), but deemed it unsuitable for forensic use based upon its state at the time [19]. Other polymer alternatives have been evaluated, such as MCLAB's NanoPOP-4™ and were deemed suitable for DNA analysis with regard to precision, accuracy, and resolution, but these products were not developed to have decreased run times [20]. Thus, this study sought to develop and validate an alternative capillary electrophoresis method suitable for forensic DNA purposes resulting in significantly quicker run times on a 3130xl using POP-6 polymer on

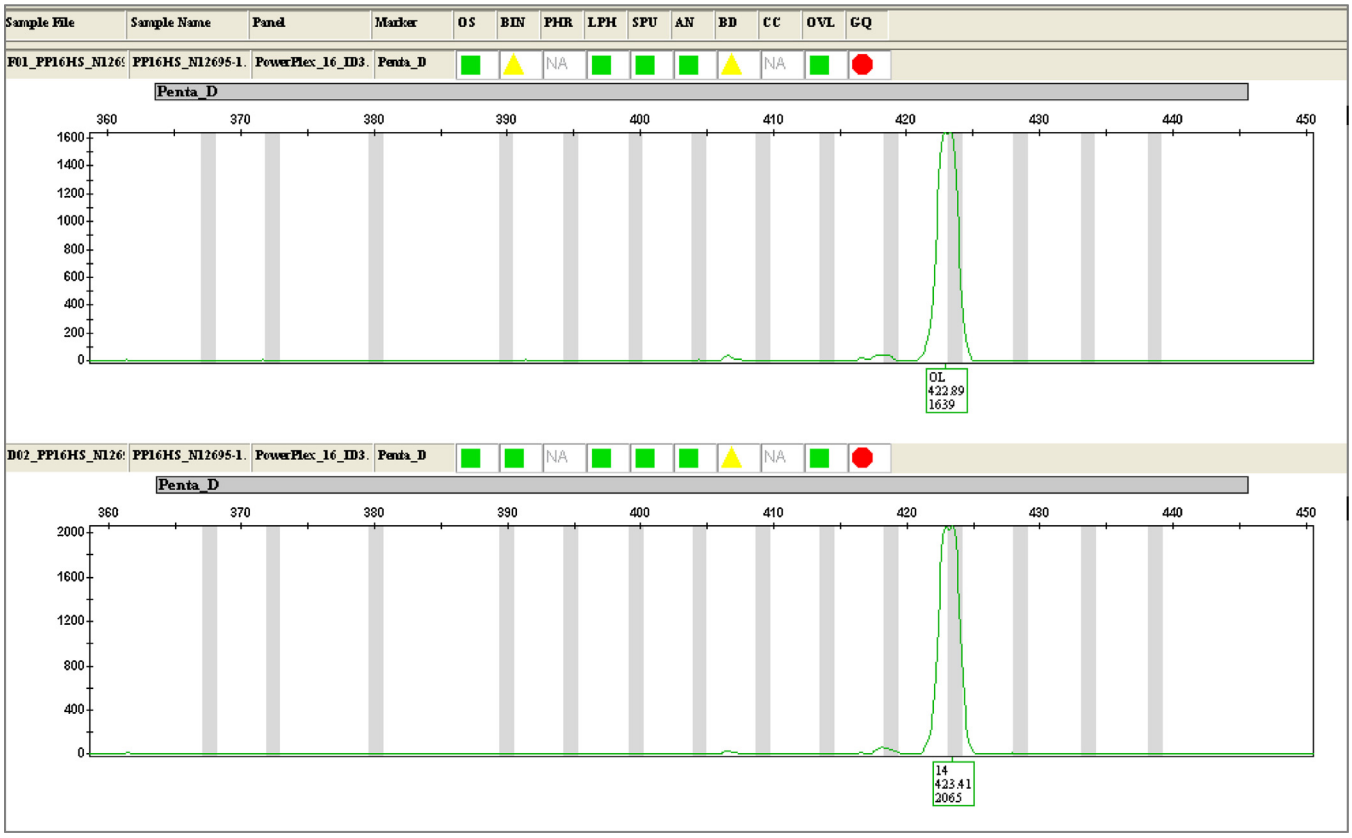

Fig. 1. One base resolution for large-sized alleles using traditional detection.
Poor resolution was obtained between alleles 13.4 ("OL") and 14 at the Penta D locus of a PowerPlex 16HS profile obtained using traditional POP-4/36 cm detection, such that both alleles repeatedly could not be detected by GeneMapper® *ID* from the same injection. The Broad Peak ("BD") quality flag was marked by a yellow triangle in the first and subsequent injections, indicating that a peak broader than what would be expected for a single allele was present. This signaled the analyst to re-process the sample. Though both alleles could never be detected by the software during the same injection (not all data shown), multiple injections did provide allele size (bases) and peak height (rfu) information for both alleles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)