Automated punching and processing of Bode Buccal 2 Cassette samples using the Hamilton easyPunch™ STARlet with the Investigator® 24plex GO! STR Kit

Dan Watsula[1], Lionel Ausset[2], Kevin Miller[3], Keith Elliott[4]
[1] Bode Technology, 10430 Furnace Road, Suite 107, Lorton, VA 22079, USA
[2] Hamilton Bonaduz AG, Via Crusch 8, 7402 Bonaduz, Switzerland
[3] Hamilton Robotics, 4970 Energy Way, Reno, NV 89502, USA
[4] QIAGEN, QIAGEN Strasse 1, 40724 Hilden, Germany

Highlights

- Easy to use Bode Buccal 2 Collectors prevent collection errors and ensure sufficient DNA is available for testing from every sample
- The Investigator 24plex GO! Kit is specifically designed for database samples and provides high first pass success rates
- Automation of both Bode Buccal 2 Cassettes and Investigator 24plex GO! Kit is achieved with no compromise on quality or performance using the Hamilton easyPunch™ STARlet system

Introduction

Since its first applications to forensic science in the 1980's, the impact of DNA testing on criminal justice has been profound. As of June 2018, fifty-six countries use DNA databases to assist in criminal investigations, with over seventy million offender profiles retained for comparison against crime scene evidence [1]. However, expanding the utility of DNA is not without challenges. In particular, the processing of such large numbers of DNA samples under stringent requirements for quality, timeliness and cost demanded by criminal justice systems represents a significant barrier for many labs tasked with handling forensic database samples.

Here we present an optimized workflow for the preparation of database samples for downstream DNA profiling using STR analysis. The workflow incorporates 1) easy-to-use sample collection devices enabling reproducible and error-free collection of DNA from individuals such as suspects, arrestees and convicted felons; 2) automation for reliable sample collection cassette manipulation, imaging, and punching while maintaining sample tracking and continuity; and 3) a DNA profiling assay optimized for direct amplification from database samples.

Bode Buccal Collectors and Cassettes

The Bode Buccal and Bode Buccal 2 DNA Collectors are easy to use, direct collection systems designed to simplify collection, improve first pass success rates and facilitate automated workflows (Figure 1). The devices utilize 100% cotton filter paper to collect a sample directly from the individual's mouth. No transfer step is required, which allows for more reliable collections and increased chain of custody control compared to cards requiring an additional transfer.

Figure 1. The Bode Buccal 2 Collector and Cassette

Hamilton easyPunch™ STARlet

The Hamilton easyPunch™ STARlet liquid handling workstation integrates sample collection card imaging, punching and liquid handling capability all in one instrument (Figure 2). The system enables full sample tracking, including the recording of card images that completely account for the consumption of evidence.

Figure 2. The Hamilton easyPunch™ STARlet

Investigator 24plex GO! Kit

The NDIS-approved Investigator 24plex GO! Kit is an expanded CODIS core loci [2] STR kit, designed specifically for direct amplification from database samples (Figure 3). The kit is highly robust ensuring excellent first pass success rates and includes QIAGEN®'s unique Quality Sensor – a quality control system that provides performance feedback for every sample, enabling the most intelligent rework strategy for any problematic samples.

Figure 3. The Investigator 24plex GO! Kit

Materials and Methods

Sample Collection

Thirty volunteer donors provided three buccal samples using Buccal 2 Collector following the manufacturer's instructions. After drying, collectors were converted and secured in the Bode Buccal 2 Cassette following manufacturer's instructions. The Cassettes were stored at ambient temperature until use and were then inserted into card magazines.

Cleaning Punch Efficiency

A cleaning punch efficiency study was carried out to determine the optimal number of cleaning punches required between samples. A cleaning punch is used to clean the punch head in between samples to prevent carryover. Sample punches from buccal cells on Bode Buccal 2 Assembled Cassettes were arranged in checkerboard patterns in 96-well plates, alternating wells containing punches from donor samples with punches from non-sample collected Bode Buccal 2 Assembled Cassettes (blanks). Samples were taken from three different donors to allow tracing of DNA carryover from the donor sample wells to the blank sample wells. Separate plates were processed to evaluate the use of zero, one or two cleaning punches.

First Pass Success Rate Study

As Bode Buccal DNA Collectors contain no added chemicals, a lysis buffer must be utilized for direct amplification reactions. The lysis buffer was prepared manually by diluting QIAGEN's Investigator STR GO! Lysis Buffer 1:5 in DNA-grade water. Additionally, Investigator 24plex GO! Kit PCR master mix was prepared manually according to the kit handbook. Diluted lysis buffer, master mix, positive control, amplification plates and card magazines were loaded onto the easyPunch™ STARlet.

The following processes were automated by the easyPunch™ STARlet instrument. After starting the script, 10 µl diluted lysis buffer was dispensed into 96-well semi-skirted PCR plates. The PCR plates were moved to the imaging platform, where the Buccal 2 Assembled Cassettes were imaged, the sampling location was determined, and the barcode sample name was read. One 1.2 mm punch was taken from the sampling area of each collection paper and was placed into an individual well of a PCR plate containing lysis buffer. One 1.2 mm punch from the cleaning area of the Buccal 2 Assembled Cassettes was taken between samples. The clean punches are discarded in an on deck waste container. The easyPunch™ STARlet imaging software analyzed each plate well immediately after punching to verify that the sample was successfully placed into the well. After punching was completed for all samples, the PCR plate was transferred to an on-deck Hamilton Heater Shaker for heated lysis at 95°C for 14 minutes. Then, the PCR plates were transferred back to the starting position and 20 µl master mix was added to each sample-containing well. From start to finish, one full 96-well plate of samples was completed in under 2 hours.

PCR plates were manually sealed and briefly centrifuged off deck to ensure all punches were fully submerged in the reaction mix. Assay plates were amplified using a GeneAmp™ PCR System 9700 thermal cycler. All PCR

reactions were performed according to the manufacturer's kit handbook using 27 amplification cycles. Lastly, capillary electrophoresis was performed using an Applied Biosystems® 3500XL Genetic Analyzer, and data analysis was performed with Applied Biosystems GeneMapper® ID-X Software v1.5. A peak detection threshold of 60 RFU, an analytical threshold of 100 RFU, and a stochastic threshold of 600 RFU were used. The acceptance criteria for heterozygous balance was set at 60%.

Results and Discussion

Cleaning Punch Efficiency Study

The electropherograms resulting from checkerboard processing were examined at each locus for indications of DNA carryover from a previous test sample. When no cleaning punch was used, DNA carryover was observed above the analytical threshold in the following blank sample. No peaks above the analytical threshold were observed using either one or two cleaning punches. One cleaning punch successfully prevented DNA carryover in over 99.5% of the 2,000+ loci analyzed; however, one blank using one cleaning punch contained three peaks between the peak detection and analytical thresholds. For operational processing, one cleaning punch is recommended as it provides the best balance between minimizing the risk of DNA carryover and laboratory processing time.

First Pass Success Rate

All samples yielded complete profiles meeting profile interpretation requirements for analytical, stochastic, and heterozygous balance thresholds without the need for reprocessing. The average peak height for an individual profile was within the optimal range of the 3500XL Genetic Analyzer at 2,775 RFU. The average profile peak height obtained per donor is displayed in Figure 4 below. The error bars on the graph represent one standard deviation from the mean.

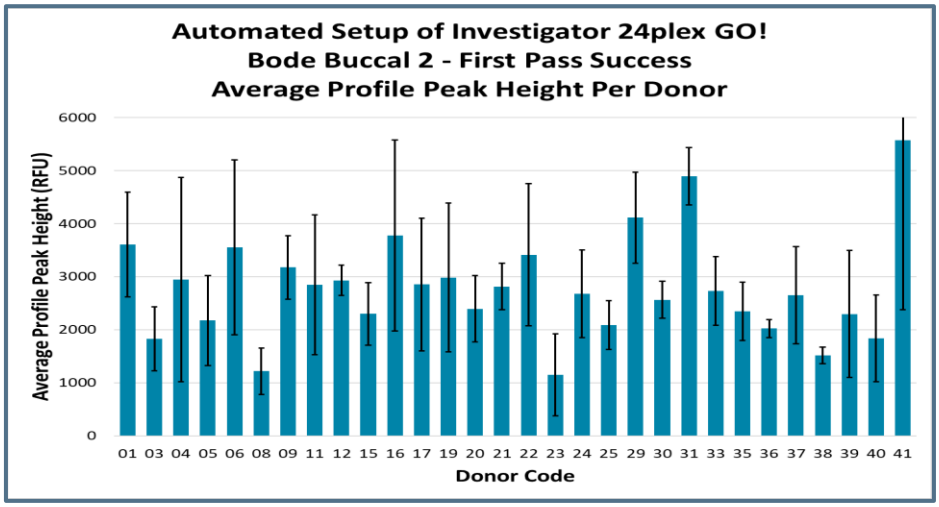

Figure 4. Average profile peak heights per donor

Intra-color balance (ICB)

Intra-color balance (ICB) is an analysis metric measuring the uniformity of loci peak heights within each dye channel. A low ICB, less than 30% for most direct amplification chemistries, can indicate problems with the assay (e.g. inhibition). The intra-color balance (ICB) was calculated by dividing the minimum locus average peak height by the maximum locus average peak height per individual color channel. The average ICB values per color channel were all greater than 50% with four out of the five channels over 60% indicating good quality profiles without any observed inhibition (Figure 5). The observed ICB values from Buccal DNA Collectors automatically processed using the easyPunch™ STARlet are consistent with the values obtained through manual setup (data not shown).

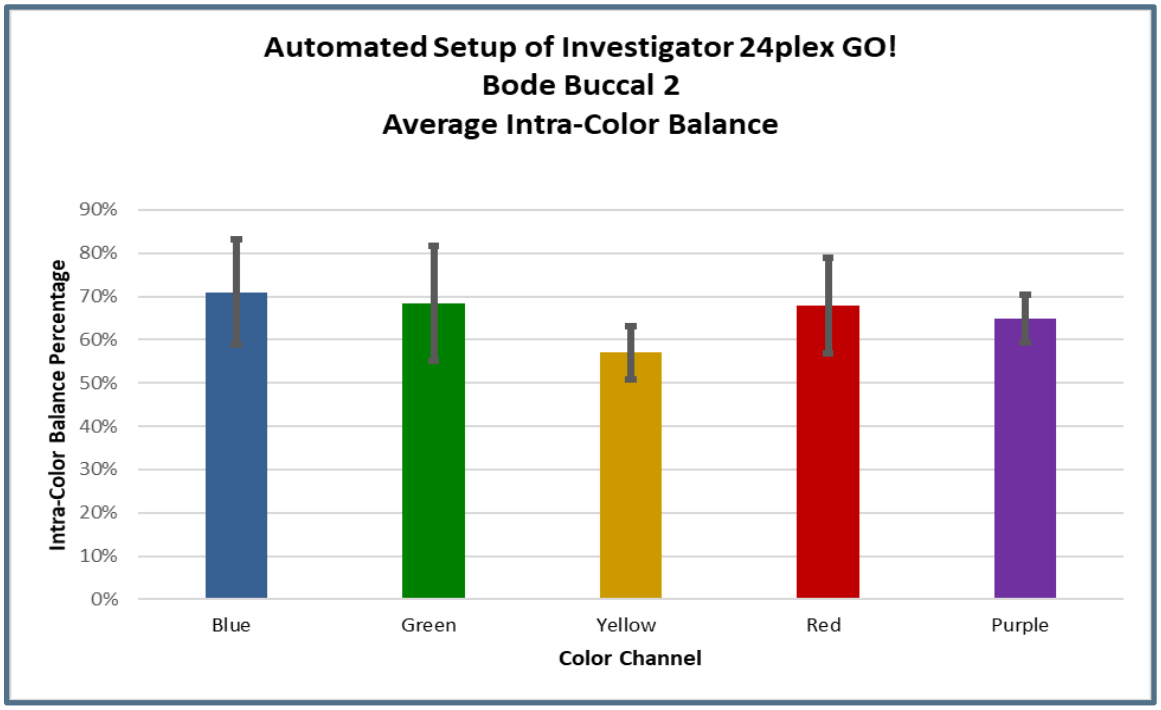

Figure 5. The average ICB across all samples.

Conclusions

Although database samples contain relatively large amounts of high quality, amplifiable DNA, scaling up throughput to enable processing of high sample volumes presents several key challenges for law enforcement and laboratories. Sample collection methods used by law enforcement officers need to be simple and effective to avoid submission of poor quality samples, and the collected samples need to be amenable to processing with automation, particularly during sample punching and assay setup. Similarly, assays need to be robust and support high first pass success rates.

Here we have described a comprehensive workflow that alleviates the key challenges within a high-throughput database workflow by integrating a robust collection product and DNA profiling assay with reliable and accurate automation for the handling of these two components.

This workflow represents an effective and scalable high-throughput solution for DNA databasing in human identity and forensics.

References

[1] DNA Resource.com http://dnaresource.com/resources.html
[2] Hares DR (2015). Selection and implementation of expanded CODIS core loci in the United States. *Forensic Sci. Int. Genetics* 17:33-34.

For More Information:
1.866.263.3443 or +1.703.646.9740
Bode.Service@Bodetech.com
www.bodetech.com