Application Note

High-throughput assay setup for the Investigator® 24plex GO! Kit from Bode Buccal 2 Assembled Cassette samples using the Hamilton® easyPunch™

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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, this expansion of the utility of DNA is not without challenges. In particular, the processing of such large numbers of DNA samples to the stringent requirements for time, cost and quality 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 easy to use sample collection devices ensuring reproducible and error-free collection of DNA from individuals such as suspects, arrestees and convicted felons; automation for reliable sample manipulation, imaging and punching of these collection cassettes while ensuring full sample tracking and continuity; and 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. The devices utilize 100% cotton filter paper to collect a sample directly from the individual's mouth. There is no transfer step required, making for more reliable collections and increased chain of custody control compared to cards requiring that extra step.

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. The system enables full sample tracking, including the recording of card images that completely account for the consumption of evidence.

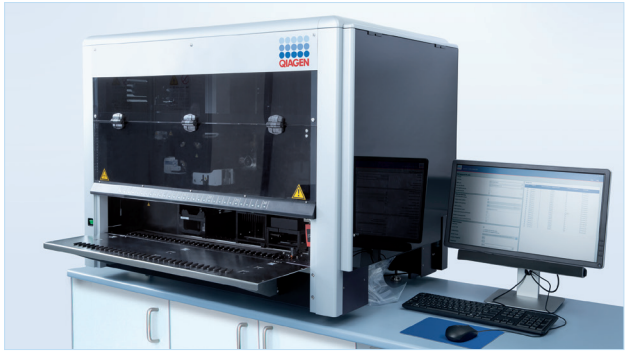

Figure 2. The Hamilton easyPunch STARlet.

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. 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

Buccal samples were collected, with informed consent, using the Bode Buccal 2 Collectors, following the manufacturer's instructions. After drying, collectors were converted and secured in the Bode Buccal 2 Cassette. The Cassettes were stored at ambient temperature until use, and were then inserted into card magazines and loaded onto the easyPunch instrument.

Protocol scripts were developed for the easyPunch STARlet to fully automate both the Bode Buccal 2 Cassette punching and the Investigator 24plex GO! Kit assay setup. As the Bode Buccal DNA Collectors contain no added chemicals, a lysis buffer must be utilized for direct amplification reactions. The lysis buffer was prepared by making a 1:5 dilution of QIAGEN's Investigator STR GO! Lysis Buffer. An aliquot of 10 µl of lysis buffer was dispensed into a 96-well semi-skirted PCR plate prior to punching the sample cassettes. Using the automated settings on the easyPunch STARlet, single 1.2 mm punches were taken from the sampling area of the collection paper and placed into individual wells of the semi-skirted plate containing lysis buffer. The easyPunch imaging software automatically analyzes each well after punching to verify that the sample is successfully placed into the well. After punching, the semi-skirted plate was transferred to an on-deck Hamilton Heater Shaker for heated lysis. Master mix was prepared from the Investigator 24plex GO! Kit according to the kit handbook and 20 µl of master mix

was dispensed into the individual wells of the 96-well semi-skirted PCR plate using the easyPunch pipetting channels and disposable tips. From start to finish, one full 96-well plate of samples was completed in under 2 hours.

A cross-contamination analysis was carried out to test for potential sample carryover. Sample punches from buccal cells on the Bode Buccal 2 Assembled Cassettes were arranged in checkerboard patterns, alternating sample wells with blank Bode Buccal 2 Assembled Cassettes. Samples were taken from different donors to allow tracing of potential sample carryover. One cleaning punch was taken from the Buccal 2 Cassette integrated clean punch paper between the cassettes.

PCR plates were sealed and 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 PCR cycles and samples were run on an Applied Biosystems® 3500xL Genetic Analyzer and analyzed with the Applied Biosystems GeneMapper® ID-X Software v1.5. For all studies, a peak detection threshold of 60 RFUs and an analytical threshold of 100 RFUs were used.

Results

First-pass success rate

Thirty volunteer donors provided three Buccal 2 Collector samples that were allowed to dry before converting to the Bode Buccal 2 Cassette. The cassettes were loaded into the magazines and processed using the optimized script protocol on The Hamilton easyPunch system. All ninety samples yielded complete profiles meeting profile interpretation requirements for analytical, stochastic, and heterozygous balance thresholds. The average peak height per allele for all ninety samples was 2,775 RFUs. The graphical representation of the average profile peak height obtained per donor is shown in Figure 4. The error bars represent one standard deviation from the mean.

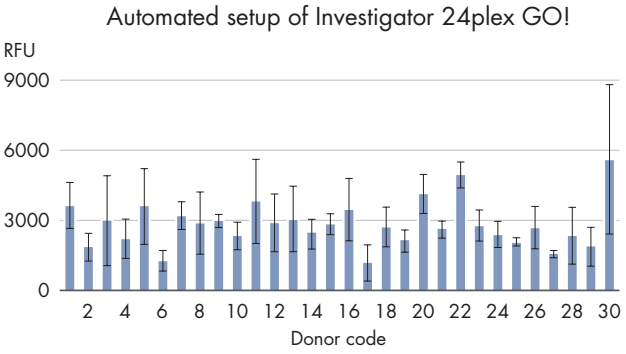

Figure 4. Average profile peak height.

The intra-color balance (ICB) is calculated by dividing the minimum locus peak height by the maximum locus peak height per individual color channel. A low ICB can indicate problems with the assay such as inhibition. The average ICB values per color channel for all ninety samples are shown in Figure 5. The average ICB values are all greater than 50% with four out of the five channels over 60% indicating good quality profiles without any observed inhibition. The error bars represent one standard deviation from the mean.

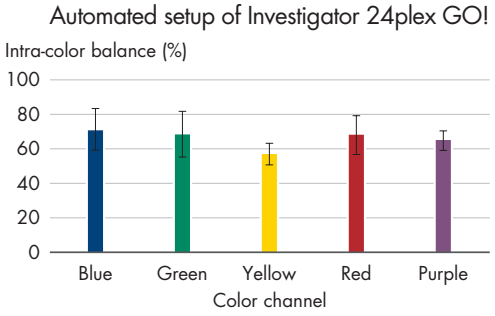

Figure 5. Average intra-color balance.

Cross-contamination study

One cleaning punch was removed from the clean punch area of the Buccal 2 Cassette prior to punching each sample. The resulting electropherograms were diligently checked for any signs of carryover from a previous test sample at each individual locus. One cleaning punch successfully prevented any signs of observed carryover in over 99.5% of the over 2,000 loci analyzed with an analytical threshold of 100 RFUs.

Conclusion

While database samples contain relatively large amounts of good quality, amplifiable DNA, scaling up throughput to enable processing of large numbers requires overcoming several key challenges. Sample collection by law enforcement officers needs to be simple and effective to avoid submission of poor quality samples, while high-throughput sample preparation can only be achieved by automated punching and assay setup. Similarly, assays should be robust in order to support high first-pass success rates. Here we have described a comprehensive workflow which integrates these key components of database workflows. Data presented here demonstrates high first-pass success rates, thanks to a robust collection system and DNA profiling assay as well as the unparalleled accuracy and reliability of automated handling of these two components. This workflow represents an effective and scalable high-throughput solution for DNA databasing in human identification and forensics.

Summary

- 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 guarantees high first-pass success rates.
- Automation of both the Bode Buccal 2 Cassettes and the Investigator 24plex GO! Kit is achieved with no compromise on quality or performance using the easyPunch system.

References

1. DNAResource.com **http://dnaresource.com/resources.html**
2. Hares, D.R. Selection and implementation of expanded CODIS core loci in the United States. Forensic Sci. Int. Genetics (2015); 17:33–34

Ordering Information

Product	Contents	Cat. no.
Investigator 24plex GO! Kit (200)*	Primer Mix, Fast Reaction Mix 2.0 including Taq DNA polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO)	382426
Investigator STR GO! Punch Buffer (200)*	Lysis buffer for 200 samples of epithelial cells on paper	386526

* Other sizes available; see **www.qiagen.com**.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.