Forensically Validated Automated Processing of FTA Cards for CODIS DNA Databasing

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Introduction

Forensic DNA Databanks are challenged with quickly processing DNA samples from arrestees, the criminally convicted, and known individuals associated with crimes for comparison against DNA profiles generated from evidence by casework analysts. Reference samples may be collected from individuals under a variety of field conditions, and therefore require collection and storage methodologies that preserve and protect DNA for future analysis.

Filter card-based sample collection methods, such as the Whatman™, EasiCollect™, and EasiCollect+ FTA filter cards from GE Healthcare Life Sciences (Marlborough, MA), simplify pre-PCR workflow steps from a variety of sample sources, and also enable ambient temperature transport and long-term storage without harm to the sample. In use, an individual's biological sample is collected and transferred to the card surface. Cells are caught in the FTA filter matrix containing chemicals that cause cellular lysis. This releases cellular DNA onto the FTA filter, thus effectively replacing the time-consuming process of DNA extraction, eliminating the need for additional equipment such as centrifuges and qPCR machines during the DNA extraction step. The cards also enable direct short tandem repeat (STR) amplification, bypassing the need for additional time-consuming steps such as quantification and normalization. Both FTA filter card types are easily integrated into a fully automated workflow for sample processing using the easyPunch STARlet assay ready workstation from Hamilton Robotics. This automated method increases reproducibility compared to manual methods while enabling analysts to refocus their attention on data analysis.

The easyPunch is based on Hamilton's Microlab® STARlet workstation platform and processes up to two 96-well microplates at once. A specialized punch module, EasiCollect or DMPK gripper arm, and light table with camera (see Deck Layout at end) are integrated into the unit, while up to four independent air displacement pipetting channels are included for high precision and reliable pipetting without reagent cross-over. Compressed O-Ring Expansion (CO-RE®) Technology creates an air-tight seal between the disposable tips and pipetting channel mandrels without using mechanical force, which maximizes sample integrity and also ensures accurate, reproducible liquid level dispensing. Barcode reading provides full sample tracking and eliminates the risk of sample mishandling or manual documentation errors. The easyPunch software is specifically pre-configured for use with EasiCollect and EasiCollect+ cards, and is qualified by Hamilton and GE Healthcare Life Sciences to optimize over 20 plate parameters and 100 card parameters when analyzing FTA cards and the corresponding processes.

Benefits-Based Highlights

- Robust, sensitive, and reproducible method to prepare nucleic acids, collected via FTA cards, for downstream amplification without sources of error and variability associated with manual processing.
- Refocus manual labor on high-value activities such as results interpretation to help alleviate laboratory bottlenecks.

The software interface guides users to enter variables such as number of cards, cleaning strikes, punch area strategy, and also allows administrators to further optimize parameters and set laboratory-specific defaults based on their validation studies. The software is linked to a database that stores plate and card images to enable traceability, prevent potential mishandling of samples and errors during punch detection, and to add photo-documentation and robotic run information to a case file.

Here, we demonstrate use of the easyPunch assay ready workstation to automate the processing of buccal and blood samples collected via FTA cards for direct amplification (see Workflow at end). In addition to the lack of contamination or sample carryover, we show that results obtained via automated methods are comparable to those obtained using manual methods while reducing active analyst time.

Materials and Methods

Buccal Sample Processing Workflow

The Wisconsin State Crime Laboratory Bureau's DNA Databank Unit performed analyses to validate and optimize the automated workflow for use in their laboratory. In this evaluation, buccal swabs were taken from 40 healthy volunteers using the EasiCollect+ buccal sample collection device. Using materials for direct amplification from the PowerPlex® Fusion 6C System[1] (P/N DC2705, Promega Corporation, Madison, WI), analysts placed a magazine containing prepared EasiCollect+ cards (P/N WB120237, GE Healthcare Life Sciences), a MicroAmp™ optical 96-well reaction plate (P/N 4316813, Thermo Fisher Scientific), positive amplification control, amplification reaction mix, and a rack of 50 µL conductive sterile filter tips (P/N 235979, Hamilton Company) on the easyPunch robotic deck. Using the filtered pipette tips, four independent pipetting channels added 12.5 µL amplification reaction mix to the reaction plate, and the plate was transferred to the punch module deck. The gripper then picked up a card and transferred it to the light table for imaging and identification, including barcode reading, determination of valid cleaning punch locations, and sample area location. The first sample card was moved in close proximity to the plate, and then one 1.2 mm sample was punched into an available microplate well, while an ionizer eliminated potential static electricity that could cause unanticipated punch sample movement. The card was returned to the magazine and the process was repeated with the remaining cards into new microplate wells. Two cleaning punches were taken from an unspotted card between each sample-containing punch in order to prevent DNA carry-over. Samples were automatically punched into the microplate wells in alternating patterns to assess cross contamination and sample carry-over between punches (Figure 1). Finally, the gripper transported the plate to its original location where 0.5 µL positive amplification control was added. A total of seven plates were processed across multiple days and alternating analysts.

After sample punching was complete, the plates were sealed, centrifuged briefly, and transferred to a GeneAmp™ PCR System 9700 (Applied Biosystems, Foster City, CA). A standard PowerPlex Fusion 6C protocol was used with 26 cycles. Amplified PCR samples were analyzed on a 3500xL genetic analyzer (Applied Biosystems), and alleles were interpreted using GeneMapper® ID-X Software version 1.4 (Applied Biosystems) with 75 relative fluorescent units (RFU) allele calling (analytical) threshold.

Whole Blood Processing Workflow

In addition to the work done at the Wisconsin State Crime Laboratory, Hamilton Company performed analyses to assess the potential for sample carry-over and compare results to manual methods. Samples consisting of 100 µL single source whole human blood from healthy, anonymous volunteers were spotted onto FTA cards from the FTA Buccal Collection Kit (P/N WB120239, GE Healthcare Life Sciences) and dried overnight. Blank cards were also prepared using 100 µL high grade distilled water. Using materials for direct amplification from the PowerPlex Fusion 18D2 (P/N DC1802, Promega Corporation), analysts placed a magazine of prepared EasiCollect cards, a MicroAmp optical 96-well reaction plate with barcode (P/N 4326659, Thermo Fisher Scientific), positive amplification control, amplification reaction mix, and a rack of 50 µL conductive sterile filter tips (P/N 235979) on the easyPunch robotic deck. Using the filtered pipette tips, four independent pipetting channels added 25 µL STR PCR master mix to the reaction plate, and the plate was transferred to the punch module deck. The gripper then picked up a card and transferred it to the light table for imaging and identification, including barcode reading, determination of valid punch locations, and sample area location. The card was then moved in close proximity to the plate, and one 1.2 mm sample was punched into an available well, while an ionizer eliminated potential static electricity that could cause unanticipated punch sample movement. The card was returned to the magazine and the process was repeated with the remaining cards into new microplate wells. One cleaning punch was taken from an unspotted card between each sample containing punch to prevent

1. *Technical Manual, PowerPlex Fusion 6C System: Instructions for Use of Products DC2705 and DC2720. Promega Corporation: Madison, WI. TMD045 Rev 6/16.*
2. *Technical Manual, PowerPlex 18D System: Instructions for Use of Products DC1802 and DC1808. Promega Corporation: Madison, WI. TMD031 Rev 5/16.*

sample carry-over. Sample punching was also performed manually for comparison purposes using the Uni-Core Punch, 1.2 mm (P/N WB100028, GE Healthcare Life Sciences). Sample plates were automatically and manually processed in triplicate. Samples were automatically and manually punched into the microplate wells in identical alternating patterns to assess cross contamination and sample carry-over between punches (Figure 2).

After sample punching was complete, positive and negative controls were added to designated wells. The plates were sealed, centrifuged briefly, and transferred to a GeneAmp PCR System 9700. A standard PowerPlex 18D protocol was used with 26 cycles. Amplified PCR samples were measured on a 3130xL genetic analyzer (Applied Biosystems) using injection conditions according to the standard PowerPlex 18D protocol, with a ten-second sample injection. Analysis was performed using GeneMapper Software version 3.2 (Applied Biosystems) with 50 relative fluorescent units (RFU) allele calling (analytical) threshold.

Results and Discussion

Buccal Sample Processing Workflow

To optimize sensitivity, precision, accuracy, and reduce or eliminate risk of contamination, the software's plate definitions and variables were set to allow a 0.5 mm gap from the bottom of the punch head to the top of the microplate well, and plate parameters in the imaging module were set to reduce or eliminate the risk of false positive punch recognition. Two cleaning punches were performed before each sample was taken in order to prevent sample carry-over between cards. FTA cards that were bent or delaminated, as well as those where the sponge applicator was not properly separated from the sample area, were manually processed.

A total of 4,017 samples were analyzed in different ways to effectively determine whether results obtained in the automated easyPunch workflow were comparable to previous studies performed manually. Figure 3 demonstrates that results obtained in the easyPunch studies closely mirror previously obtained results. In addition, comparable results were obtained across multiple samples, plates, days, and analysts. Finally, every allele typed in the study was graphed (Figure 4), where red marks indicate an allele, and a thick black line indicates the average of all alleles at the corresponding locus. This average shows that the loci across all dye channels appear relatively balanced. Additionally, the average allele RFU values tend to be approximately fifteen times the validated Stochastic Threshold (ST). Results indicate that 96% of samples yielded complete DNA profiles on the first pass (Table 1).

Whole Blood Sample Processing Workflow

The STR profiles generated show that samples isolated and prepared with the easyPunch system are identical to those prepared manually (Table 2). No cross-contamination was observed in either sample processing workflow. Some spurious peaks were detected in blank cards, but the heights of these peaks were never more than 30 RFU, whether processed by the easyPunch or manually, so were discounted as they were less than the analytical threshold. Low level contamination from the blank cards could likely be reduced by increasing the number of cleaning punches between sample punches.

Conclusion

The automated easyPunch workflow facilitates both the automated punching and analysis of biological samples, such as buccal cells and whole blood, for databasing DNA from FTA cards. Results obtained from both locations demonstrate that easyPunch is a robust and reliable system for use with direct amplification kits to standardize high-throughput STR analysis.

Table 1: First Pass Success Rates from Buccal Samples on EasiCollect+ FTA Cards

	Updated Stats	Updated Stats	Updated Stats
	Total Samples	Failed Samples	First Pass Success Rate
Pre Punch Solution	1,654	192	88%
Punch Solution (2 months)	4,017	141	96%

Table 2: STR Profiles Generated from Blood Samples on EasiCollect Cards

	Manual Punch		EasyPunch STARlet		% Cross Contamination
	Blood Sample	Blank Sample	Blood Sample	Blank Sample	–
No DNA Profile Obtained	0	126	0	126	0
Full DNA Profile Obtained	126	0	126	0	0

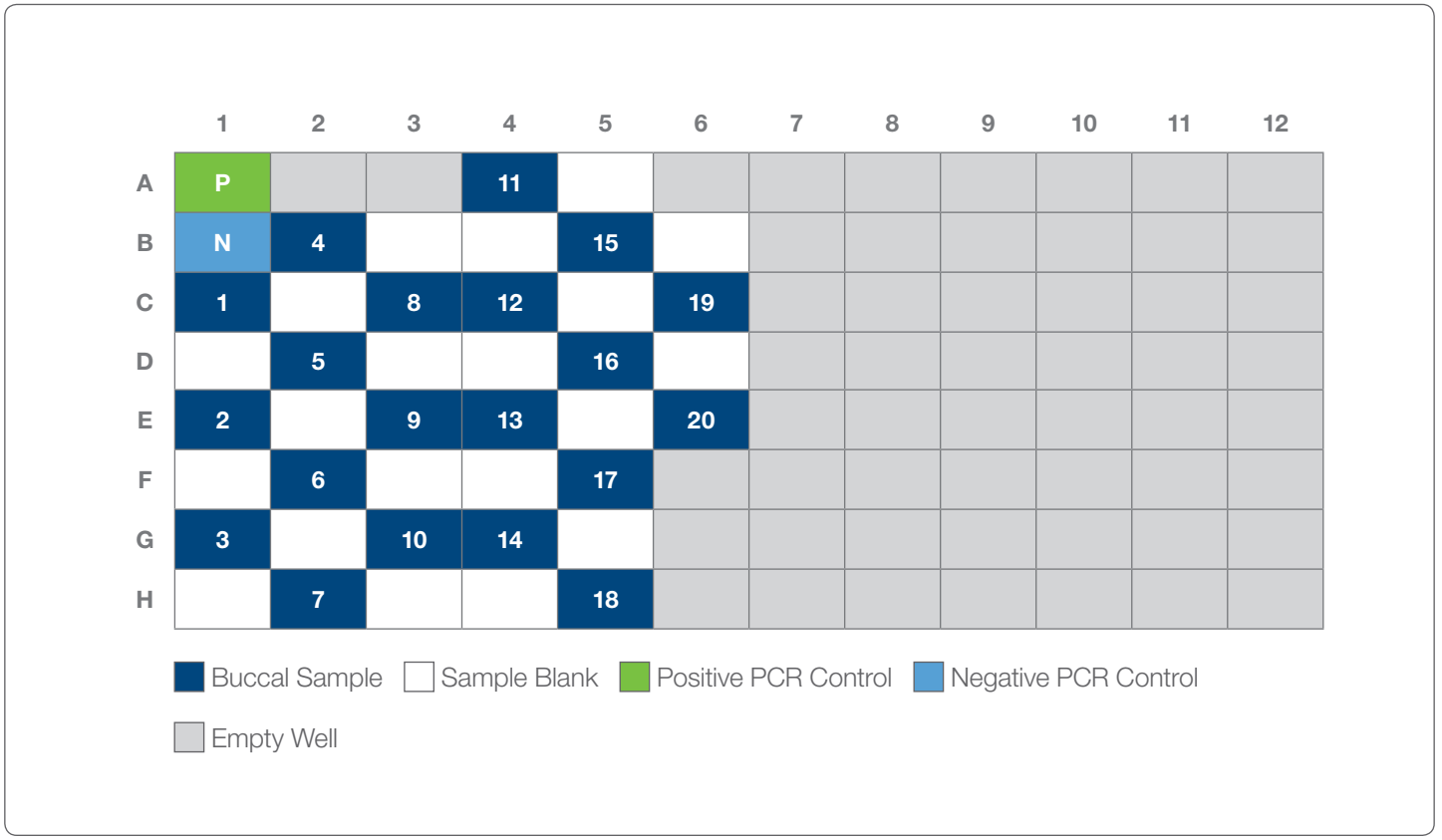


Figure 1: Wisconsin State Crime Laboratory Bureau's DNA Databank Unit PCR plate layout indicating buccal samples, sample blanks, controls, and empty wells.

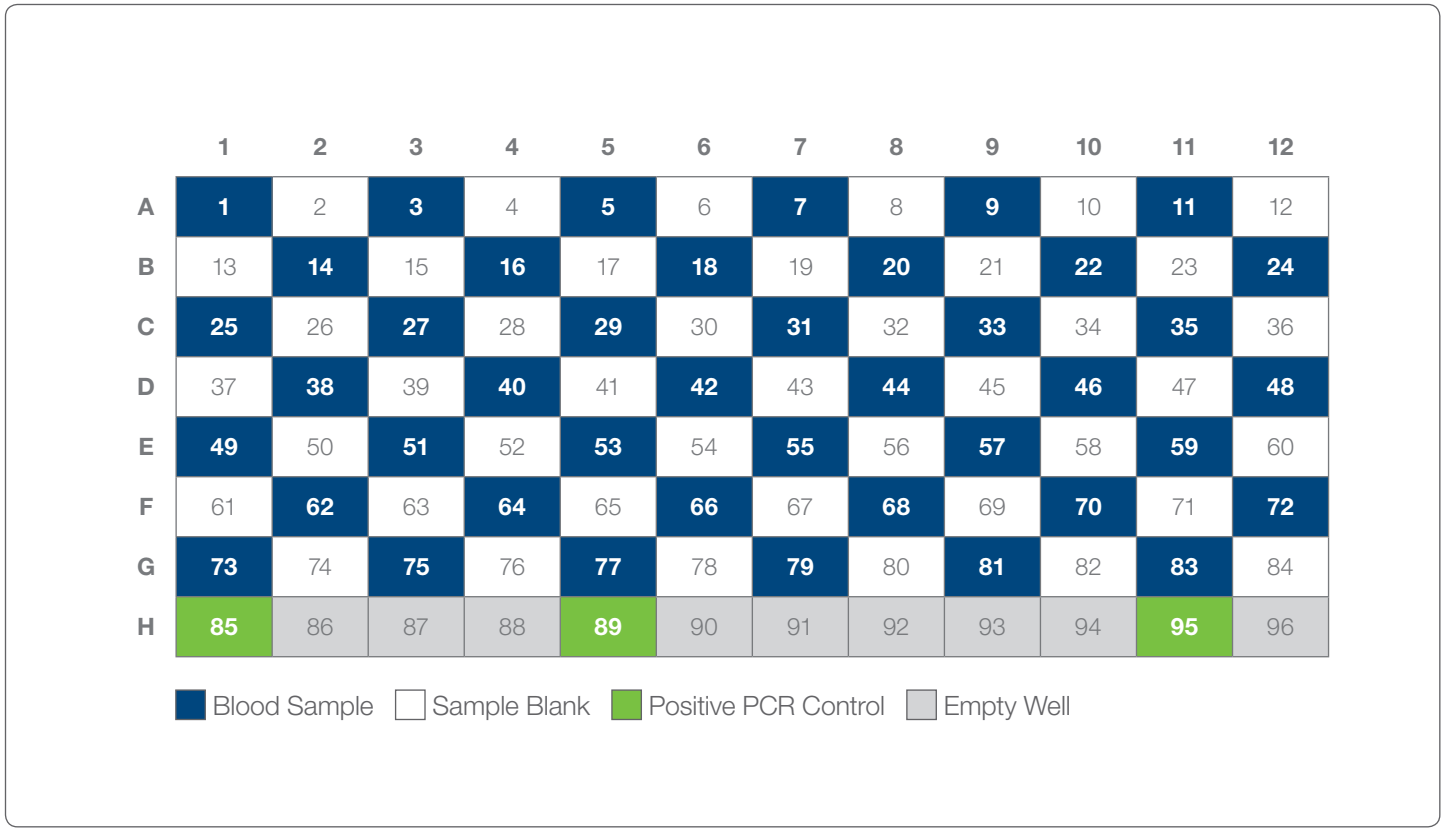


Figure 2: Hamilton Company PCR plate layout indicating blood samples, sample blanks, positive controls, and empty wells.

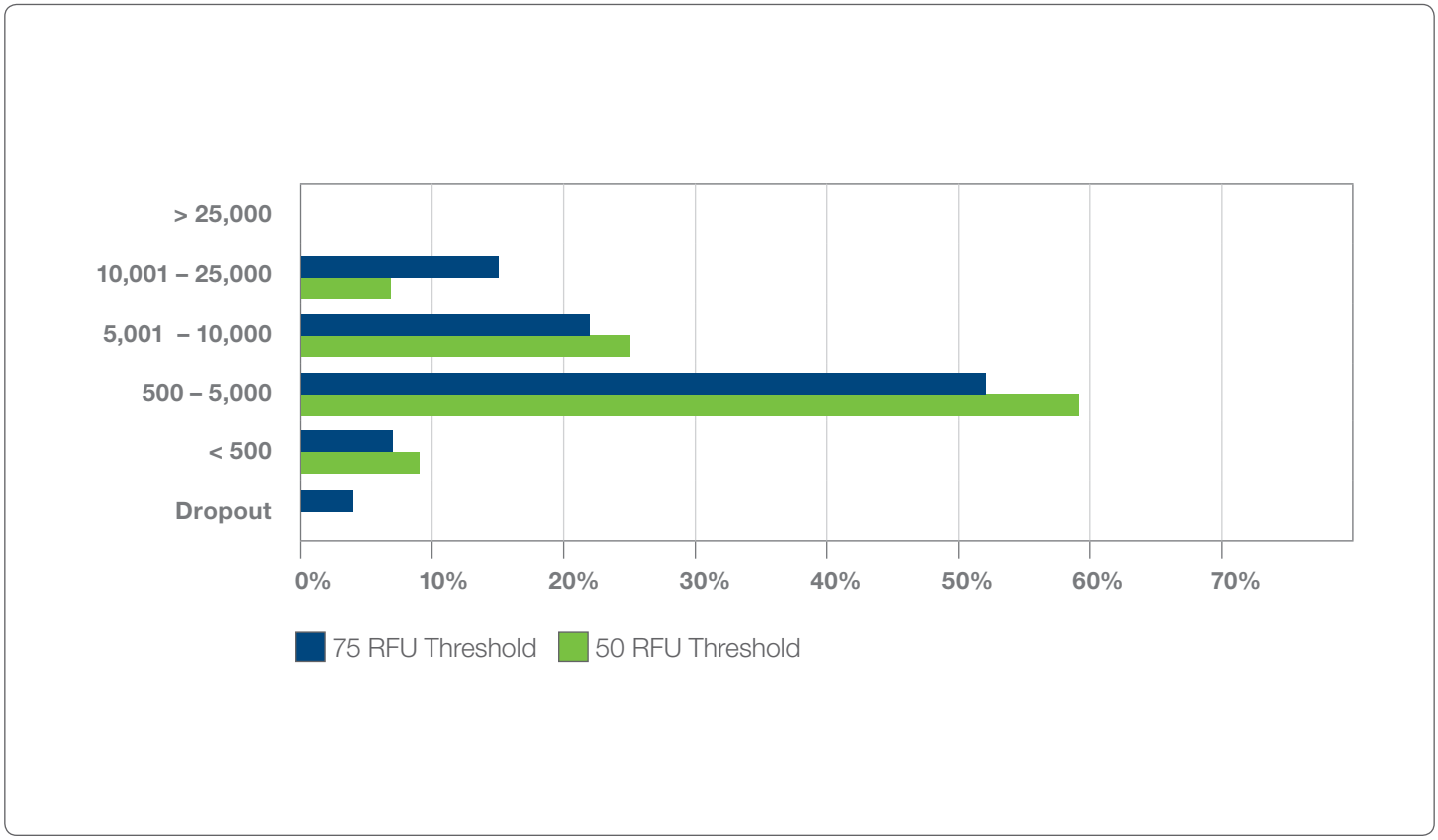


Figure 3: Comparison of average sample RFU obtained from the Wisconsin DNA Databank Unit using previously obtained results in the manual workflow (green) and the automated easyPunch workflow (blue).

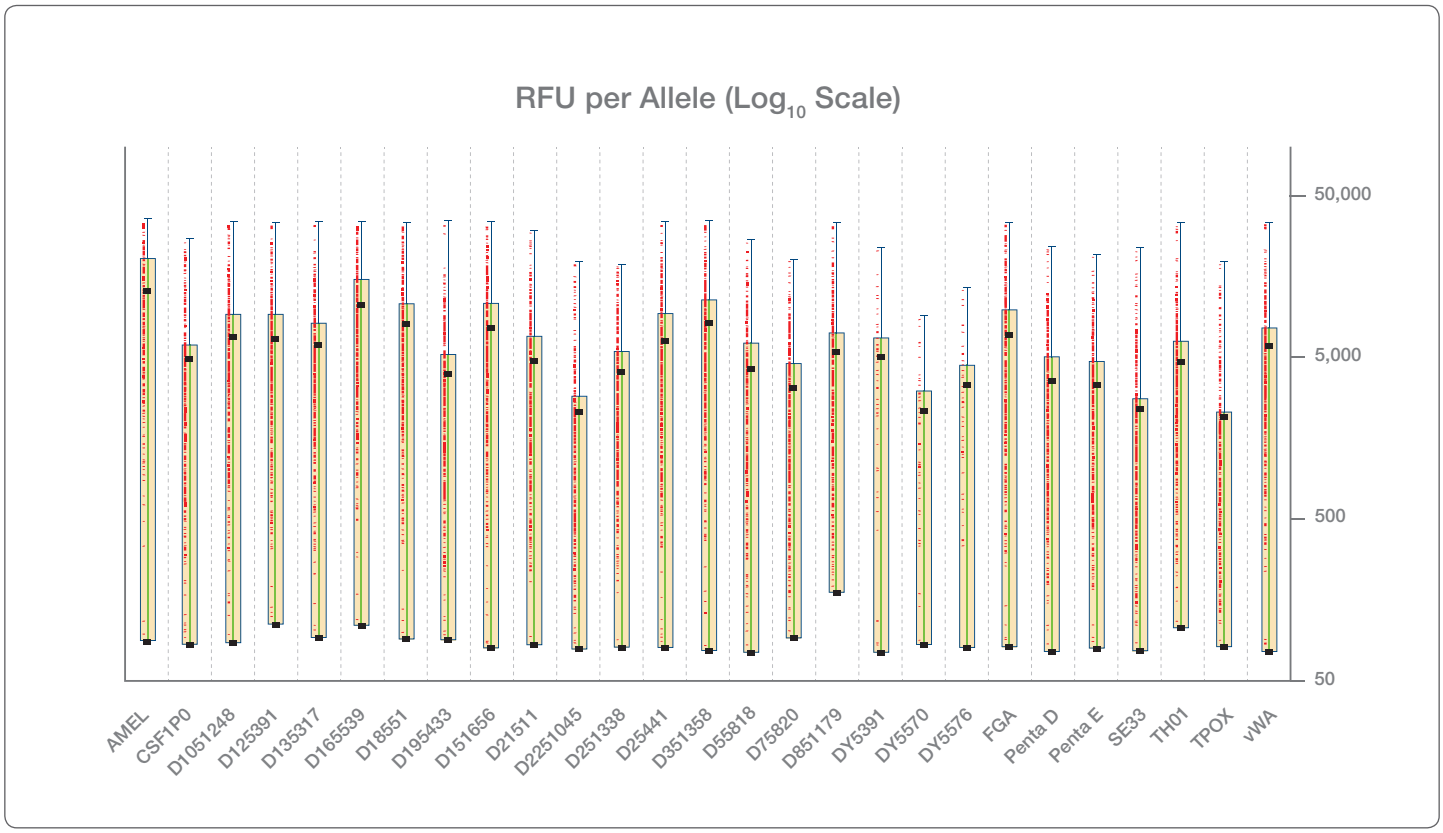
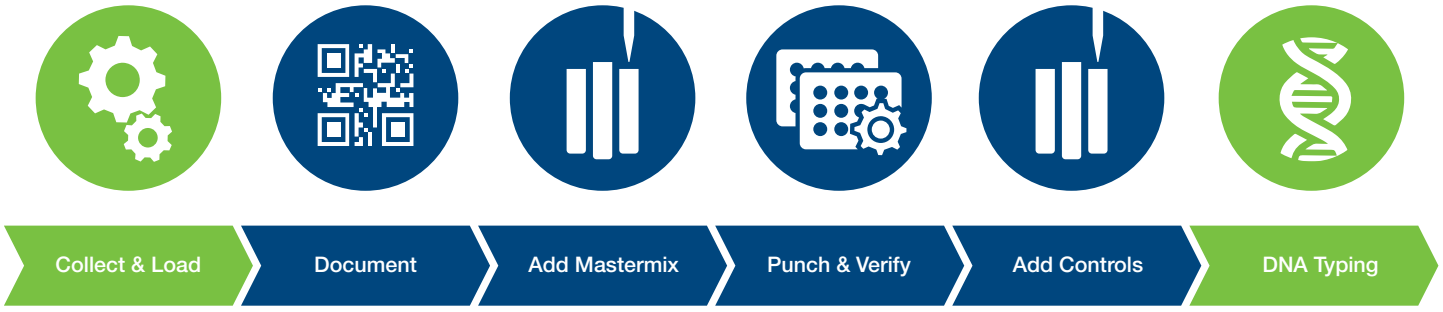
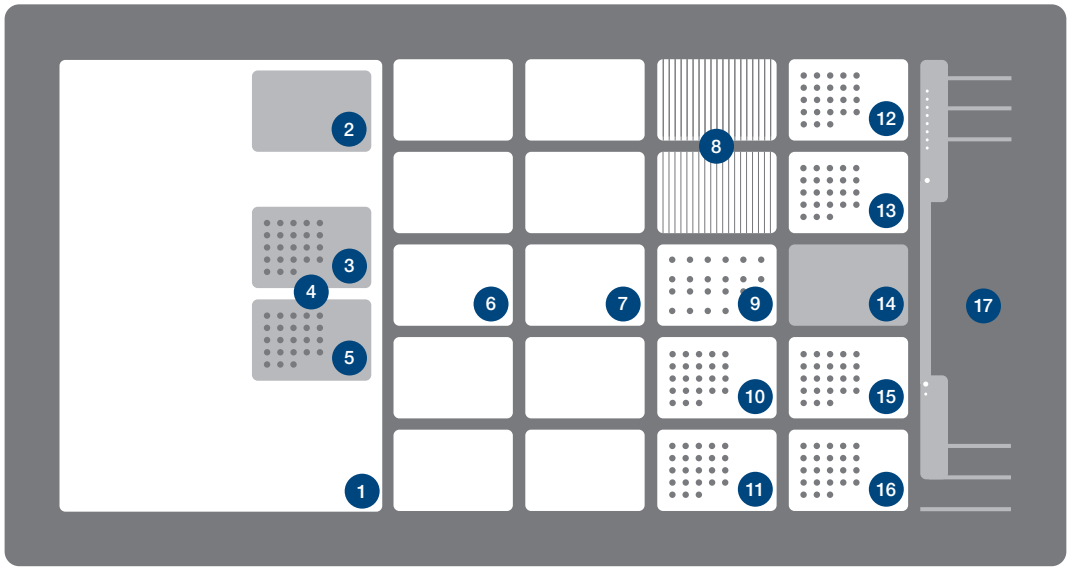


Figure 4: RFU values for each allele (red marks) and averages of all alleles at the corresponding loci (thick black line).

Workflow



EasyPunch Deck Layout



1. Light Table
2. Service Port
3. Sample Plate Position
4. Punch Waste Receptacle
5. Sample Plate Position
6. Sample Card Magazines
7. Sample Card Magazines
8. Recovery Modules
9. Reagent Tube Position
10. 96 Well PCR Plate
11. 96 Well PCR Plate
12. Variable Volume CO-RE Tip Position
13. 50 µL CO-RE Tips
14. Open Position
15. 96 Well PCR Plate
16. 96 Well PCR Plate
17. Waste

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