ACCELERATING THE STR ANALYSIS WORKFLOW THROUGH AUTOMATION AND DIRECT AMPLIFICATION METHODS

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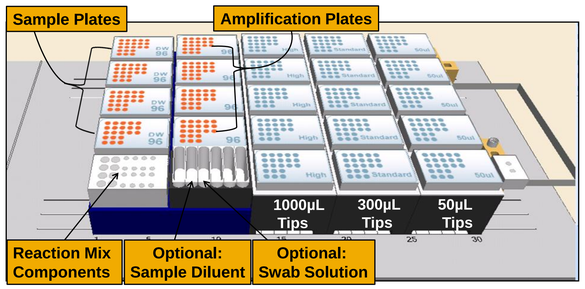
1. Introduction

Analysis of amplified short tandem repeat (STR) multiplexes remains the primary technique for human identification. Several developments in STR analysis can improve workflow efficiency, including automated amplification plate setup, and direct amplification of samples. We demonstrate automated direct amplification methods on two robotic platforms using PowerPlex® STR multiplexes. Swabs and punches from FTA® were typed with PowerPlex® Fusion, ESI 17 Fast, 18D, and Y23 Systems. Amplifications from swabs and punches were performed without sample purification or wash steps, utilizing methods on the Hamilton STARlet and easyPunch STARlet liquid handling workstations. With reliable amplification and the absence of detectable cross-contamination, the automated reaction setup provides a high first-pass success rate for direct amplification. Efficiencies can be gained through a reduction in both hands-on reaction setup time and potential handling errors, as well as barcoded sample tracking and report generation.

2. Identity Automation™ STAR-line Direct Amplification Method

Identity Automation™ Hamilton STAR-line Direct Amplification PowerPlex® reaction setup has been developed on the Hamilton STARlet (8 Channel, AL). The method features a flexible structure that allows customizable instrument and deck configurations including both hardware and labware.



- Worklist driven execution featuring Promega's STR Normalization Manager™
- Sample and plate barcoding; including worklist match
- Multi-plate setup with plate map flexibility
- On deck reaction mix creation from component tubes
- Flexible sample input (tubes and plates)
- SwabSolution™ reagent or punching buffer dispensing (optional)
- Single or multi-dispense reaction mix delivery
- CE import file creation

2a. Adaptable Configuration

- Install on STARlet, STAR, STARplus
- Load labware Manually or with Autoload
- Utilize carriers and labware that match your overall workflow
- Choose your desired deck layout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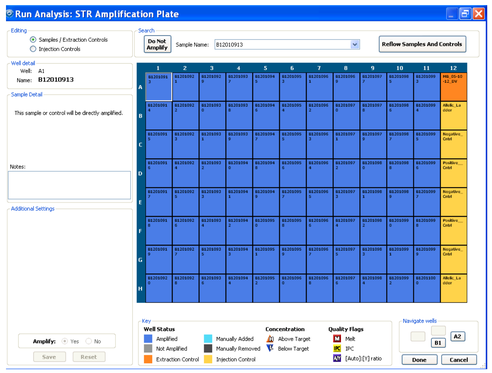
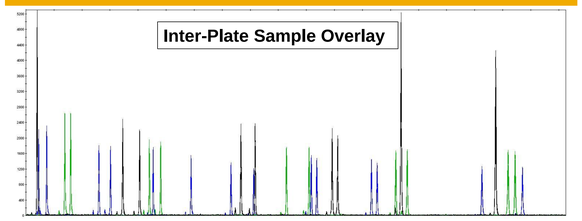
2b. Barcode Tracking and Flexibility

- Load sample plates or tubes in any order
- Missing sample or plate notification via deck-to-worklist barcode matching
- Carrier and tip type/position verification (Autoload)



The STR Normalization Manager™ software is a user-friendly, integrated run configuration application, offering the ability to create assay and plate templates, with complete flexibility and detailed report files. Additional output files include a CE import file, as well as a pipetting worklist for seamless execution.

2c. Multi-Plate Setup



PowerPlex® Fusion half-volume reactions: Instrument-prepared reaction mix, multi-dispensed into four replicate amplification plates. The overlay above shows eight profiles (3 channels) in four pair-wise swab-extract replicates (A1 & A12) across four amplification plates. The profile trend shows reproducible amplification across plate columns and between amplification plates.

2d. Identity Automation™ STAR-line Direct Amplification of Swab Extract

Replicate OmniSwab™ buccal samples were taken from 10 individuals, and incubated in SwabSolution™ reagent for 30 minutes at 70°C. Replicate samples were pooled and dispensed into 2.2-mL deep-well plates in one of two layouts: **A)** Pool replicates across plate row wells (12), or **B)** Alternating Male/Negative checkerboard pattern. Plates A and B were utilized for multiple automated runs.

Source Plate	STR Multiplex	Amp Plate Replicates	Amplifications	Setup Time	Automated Run Time	% First Pass Full Profiles
A	ESI 17 Fast	1	88	5 min	20 min	100%
A	Fusion	2	176	6 min	29 min	100%
A	Fusion	4	352	7 min	58 min	100%
B	Y23	1	42	5 min	20 min	100%

All 658 amplifications produced full profiles, while all negative amplifications were free of STR peaks. Each run required less than 10 minutes of hands-on setup time, with 4 plates being prepared by the instrument in just under 1 hour.

2e. Identity Automation™ STAR-line Direct Amplification of FTA®

Buccal swab samples were transferred onto FTA® storage cards, and manually punched (1.2mm) into one of two amplification plate checkerboard layouts containing 5-µl of water pre-dispensed by the automated platform. Negative amplifications received no punch, and cleaning punches were performed between samples. PowerPlex® ESI 17 Fast, Y23, and Fusion 25-uL amplifications were prepared with several automated runs.

Number of Runs	Amp Plates Per Run	Total Amplifications	Run Setup Time	Automated Run Time	% First Pass Full Profiles
3	1	126	5 min	38 min	99.2%

125 out of 126 amplifications produced full profiles, while all negative amplifications were free of STR peaks. 23/24 loci were called for one punch sample, which was attributed to poor sample transfer at the card location punched. Each run required approximately 5 minutes of hands-on setup time (not including manual punch time), with 1 plate being prepared by the instrument in just under 40 minutes. Longer run time is attributed to single dispense, fresh tip reaction mix delivery.

3. easyPunch HID Direct Amplification Method

The easyPunch STARlet consists of a STARlet Autoload liquid handling workstation comprising 4 independent 1 ml channels, a card and plate gripper and the punch module. The system and HID method features automated sample card punching and reaction mix delivery on one platform.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- Barcoded worklist or loaded card order execution
- Input and output file can be tailored for downstream systems and LIMS integration
- Reaction mix delivery

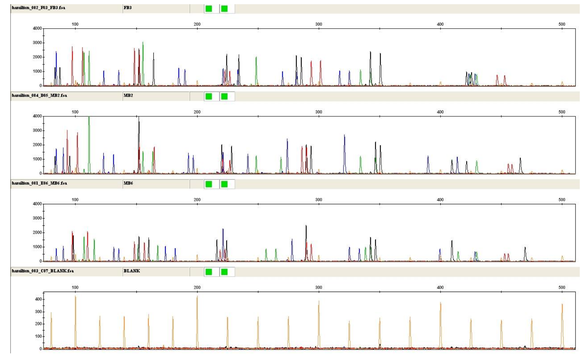
3a. easyPunch HID Direct Amplification of FTA® Blood and Buccal Swab Transfers

Whole blood and buccal swab samples were transferred onto FTA® storage cards. Hamilton's HID method was utilized to distribute reaction mix and punch each card (1.2mm) into amplification plates according to a worklisted checkerboard layout.

Run	STR Multiplex	Reaction Volume (µL)	Amp Plate Replicates	Amplifications	% First Pass Full Profiles
1	PowerPlex® Fusion	25	1	60	100%
2	PowerPlex® Fusion	12.5	1	60	100%
3	PowerPlex® 18D	25	1	60	100%
4	PowerPlex® 18D	12.5	1	60	100%
5	PowerPlex® Y23	25	1	60	100%
6	PowerPlex® Y23	12.5	1	60	100%

All 360 amplifications produced full profiles, while all negative amplifications were free of detectable STR peaks. Each run required approximately 20 minutes of hands-on setup time, including manual reaction mix creation. The HID method was able to prepare/punch one plate (60 punches) in approximately 50 minutes with one cleaning punch between samples. A full plate of 96 punches can be prepared in approximately 75 minutes.

3b. easyPunch HID Direct Amplification of Blood on FTA®



Panels 1-3 above exhibit profiles (five-color) of three independently collected, punched, and amplified blood samples on FTA® using PowerPlex® Fusion. The bottom panel shows a negative amplification (five-color) with no detectable STR peaks. One cleaning punch between samples proved to be sufficient in preventing detectable cross-contamination.

4. Conclusions

As the demand for STR analysis increases, there is a growing need to increase sample throughput and provide reliable sample tracking during analysis. Utilizing the described automated direct amplification methods can help satisfy these growing needs.

Identity Automation™ STAR-line Direct Amplification Method:

- STR Normalization Manager™ maintains complete run-by-run flexibility
- Less than 10 minutes of hands-on time to setup 4 direct amplification plates
- Reduces potential handling errors
- Fast automated run times
- Highly reproducible data
- Maintain data integrity via sample/plate barcoding
- No cross-contamination
- CE import and report file creation

easyPunch HID Direct Amplification Method:

- Reduces hands-on time by combining card punching and reaction setup on one system
- No detectable cross-contamination when performing 1 cleaning punch between samples
- Efficient sample spot detection and accurate punching
- File input and output can be tailored to downstream systems or LIMS

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