Application Note

Fully Automated Punching and Setup for Direct Amplification of FTA® Card Samples Using QIAGEN Investigator® STR GO! Kits on the Hamilton® easyPunch™ STARlet Workstation

Laurent Baron[1], Lois Tack[2], Keith Elliot[3], and Mario Scherer[3]
[1] Hamilton Bonaduz AG, Bonaduz, Switzerland
[2] Hamilton Robotics, Inc., Reno, Nevada, USA
[3] QIAGEN GmbH, Hilden, Germany

Introduction

In generating today's reference databases, FTA and other paper card substrates are often used for sample collection, due to the ease of storing and shipping. However, the effort needed to punch and prepare these FTA cards for amplification, while ensuring sample ID traceability, represents a significant bottleneck for processing reference samples. Here we describe a new workflow for streamlined processing of such samples, using QIAGEN Investigator STR GO! Kits and the Hamilton easyPunch STARlet workstation. This workflow enables fully automated sample punching and PCR setup of up to 96 samples in around 90 minutes.

QIAGEN's portfolio of direct amplification kits for STR analysis includes three innovative formats: Investigator 24plex GO! Kits for the new expanded CODIS set, Investigator IDplex GO! Kits for the CODIS markers, and Investigator ESSplex SE GO! Kits for the European Standard set with SE33. Here we describe validation of sample punching and PCR setup for Investigator IDplex GO! and Investigator ESSplex SE GO! Kits on the Hamilton easyPunch STARlet.

Methods

EasyPunch protocol scripts were developed to fully automate both FTA sample card punching and STR assay plate setup. Master mix was prepared from the Investigator IDplex GO! and the Investigator ESSplex SE GO! Kits. Separate easyPunch methods were developed to process buccal cell stains on FTA cards with indicator (Whatman® EasiCollect™, GE Healthcare), or blood spots on FTA cards (Whatman). A single punch, 1.2 mm in size, was taken from the center of each sampling area on each card into a well of a 96-well PCR plate (Bio-Rad® Hard-Shell®). Before punching, 25 µl ▷

of PCR master mix prepared from the Investigator kit reagents was dispensed into the plate using the easyPunch pipetting channels and disposable tips. Samples from the same donors were processed manually in parallel. The easyPunch imaging software was activated to analyze each plate well after punching.

Blood from 100 different donors and buccal cells from 38 different donors were collected onto FTA cards. Each card was punched in replicates of 5 using both Investigator IDplex GO! and Investigator ESSplex SE GO! Kits. PCR plates were sealed and centrifuged to ensure all punches were fully submerged. Assay plates were amplified using an Eppendorf® Mastercycler® ep thermal cycler. PCR was performed according to the corresponding kit handbooks, using 25 PCR cycles for blood samples and 28 cycles for buccal cell samples. Samples were run on an Applied Biosystems® 3500 Genetic Analyzer and analyzed with Applied Biosystems GeneMapper® ID-X Software v1.2 using a 100 RFU allele-calling threshold.

A cross-contamination analysis was carried out to test for potential sample carryover. Sample punches from buccal cells on FTA cards were arranged in checkerboard patterns, alternating sample wells with blank card punches. Samples were taken from different donors to allow tracing of potential sample carryover. Three cleaning punches were taken between different cards. Samples were amplified using the Investigator ESSplex SE GO! Kit for 28 PCR cycles. Data were analyzed with the Applied Biosystems GeneMapper ID-X Software v1.2 using a threshold of 30 RFU for detection of alleles in empty card samples.

Results

Full DNA profiles were obtained for all samples. Example STR profiles are shown in figures 1–4.

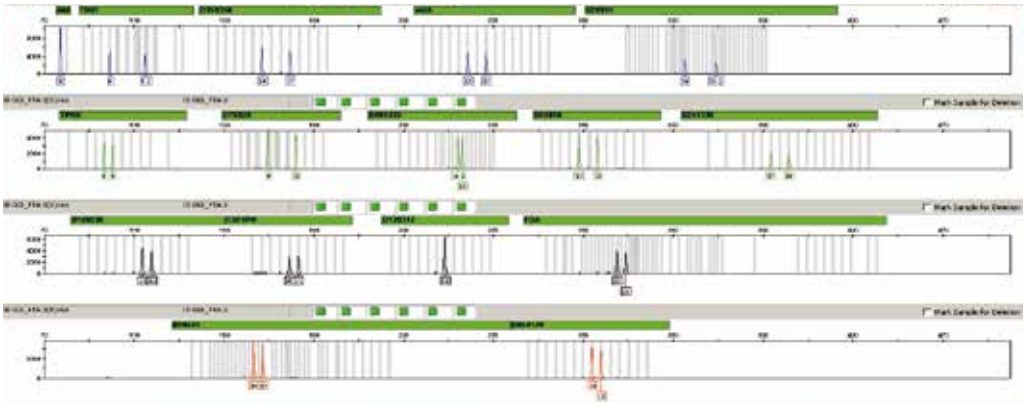

Figure 1. Example STR profile for a blood spot on an FTA card, amplified with the Investigator IDplex GO! Kit. Data analysis was performed using Applied Biosystems GeneMapper ID-X Software.

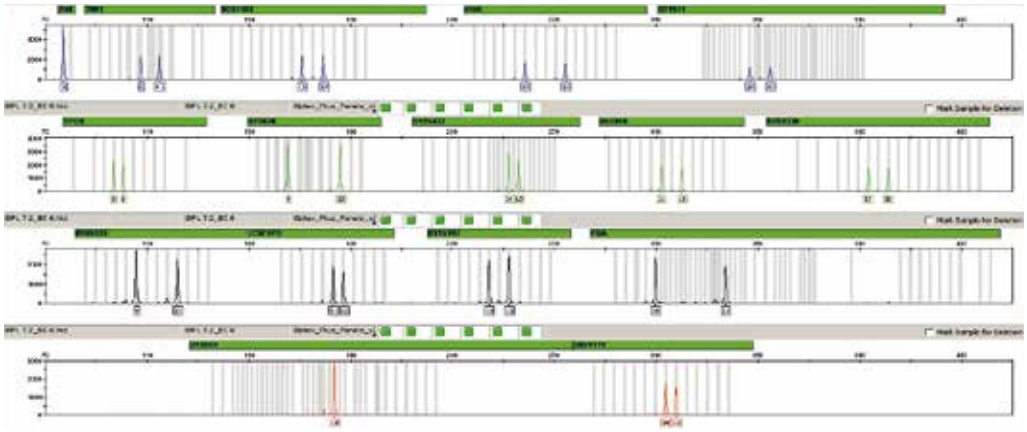

Figure 2. Example STR profile for a buccal cell stain on an FTA card, amplified with the Investigator IDplex GO! Kit. Data analysis was performed using Applied Biosystems GeneMapper ID-X Software.

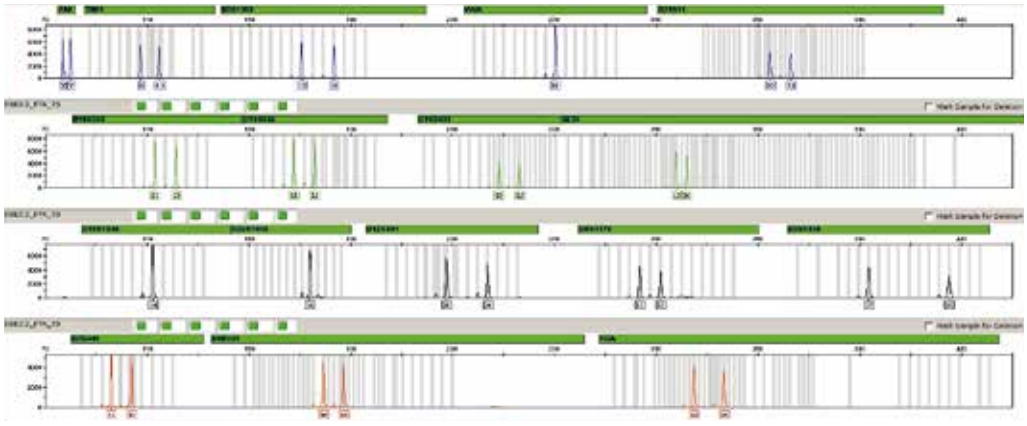

Figure 3. Example STR profile for a blood spot on an FTA card, amplified with the Investigator ESSplex SE GO! Kit. Data analysis was performed using Applied Biosystems GeneMapper ID-X Software.

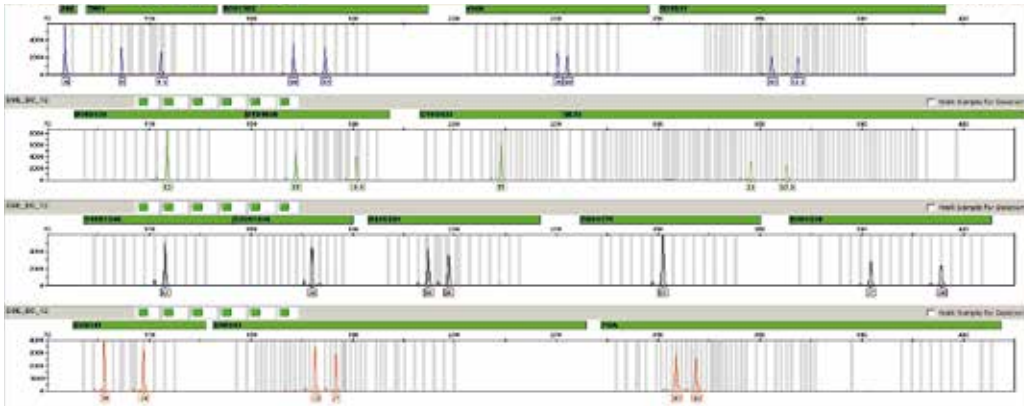

Figure 4. Example STR profile for a buccal cell stain on an FTA card, amplified with the Investigator ESSplex SE GO! Kit. Data analysis was performed using Applied Biosystems GeneMapper ID-X Software.

Visual inspection of the PCR plate confirmed that a single punch had been transferred to each well, as expected based on imaging analysis of the plate after each punch cycle. No incidents of sample mix-up were observed. Results obtained using the automated easyPunch method were comparable to results for manually processed samples from the same donors.

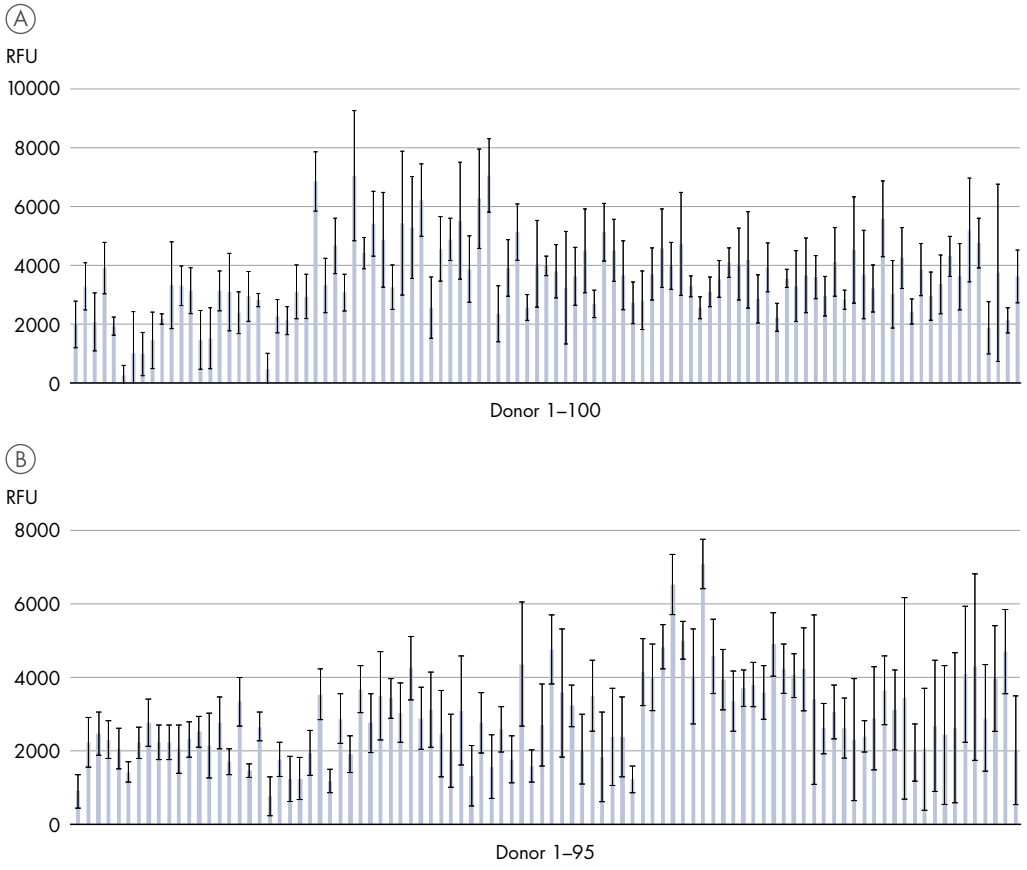

Figure 5. Analysis of blood on FTA card samples. A: Average peak heights for samples from 100 different donors processed in 5 replicates are shown. Samples were amplified and analyzed using the QIAGEN Investigator IDplex GO! Kit, according to manufacturer's instructions. **B**: Average peak heights for samples from 95 different donors processed in 5 replicates are shown. Samples were amplified with the QIAGEN Investigator ESSplex SE GO! Kit.

Average peak heights following PCR amplification of all blood and buccal cell samples are shown in figures 6 and 7.

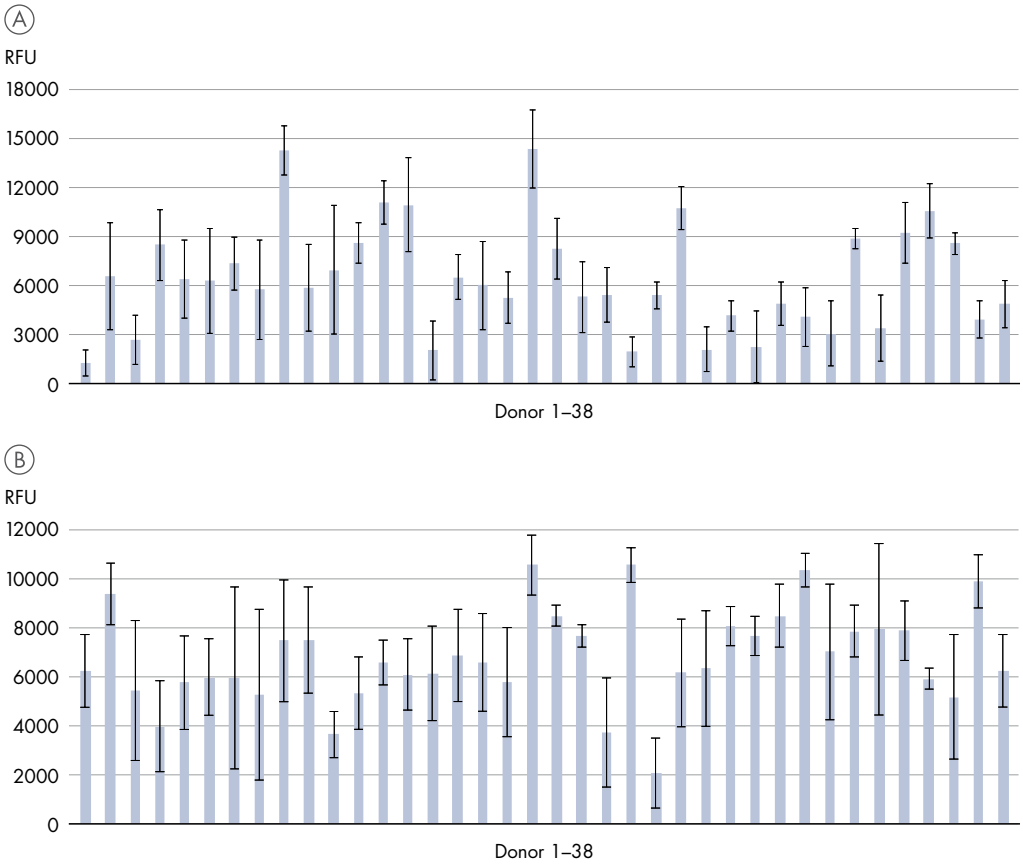

Figure 6. Analysis of buccal cells on Whatman EasiCollect cards. Average peak heights for samples from 38 different donors processed in 5 replicates are shown. Samples were amplified using **A**: the QIAGEN Investigator IDplex GO! Kit or **B**: the Investigator ESSplex SE GO! Kit. Varying signal heights are typical for buccal cell samples.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PCR+	NEG	7	NEG	14	NEG	PCR+	NEG	7	NEG	14	NEG
B	NEG	4	NEG	11	NEG	18	NEG	4	NEG	11	NEG	18
C	1	NEG	8	NEG	15	NEG	1	NEG	8	NEG	15	NEG
D	NEG	5	NEG	12	NEG	19	NEG	5	NEG	12	NEG	19
E	2	NEG	9	NEG	16	NEG	2	NEG	9	NEG	16	NEG
F	NEG	6	NEG	13	NEG	20	NEG	6	NEG	13	NEG	20
G	3	NEG	10	NEG	17	NEG	3	NEG	10	NEG	17	NEG
H	E	E	E	E	E	E	E	E	E	E	E	E

ESSplex SE GO! IDplex GO!

Figure 7. Setup of cross-contamination study. Positions of buccal cell samples, empty cards, positive PCR control and empty wells are indicated. **NEG**: empty FTA card, **1–20**: FTA card donor 1–20, **PCR+**: positive PCR control, **E**: empty position for allelic ladder.

For the cross-contamination analysis, samples were arranged as shown in figure 7. No incidences of sample carryover were observed (figures 8 and 9).

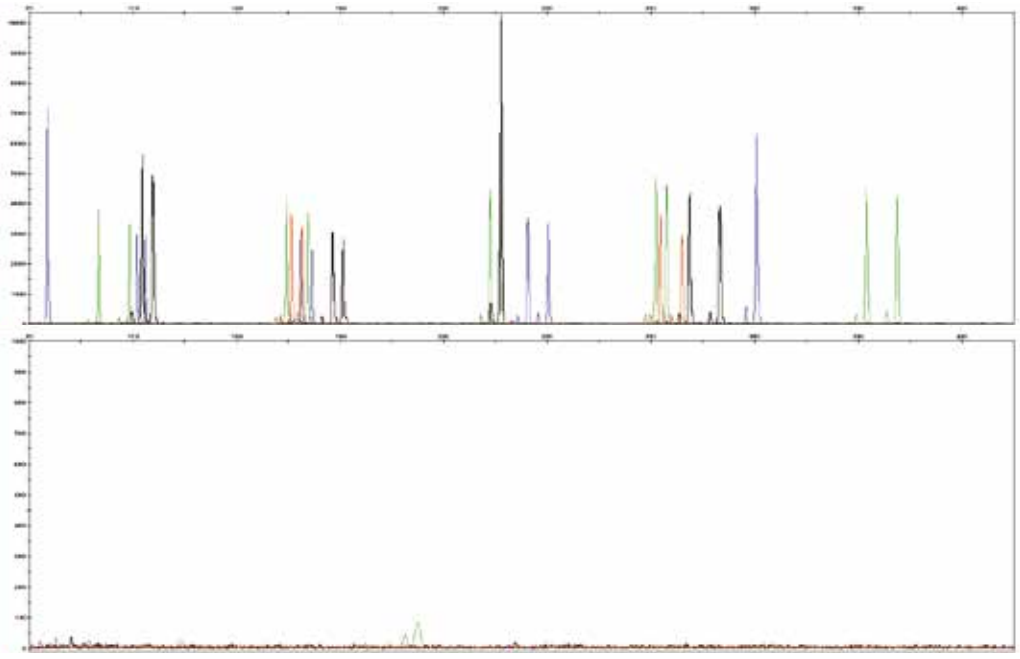

Figure 8. Cross-contamination analysis. Examples of typical sample electropherograms for positive (upper panel) and negative (lower panel) sample punches are shown for the Investigator IDplex GO! Kit.

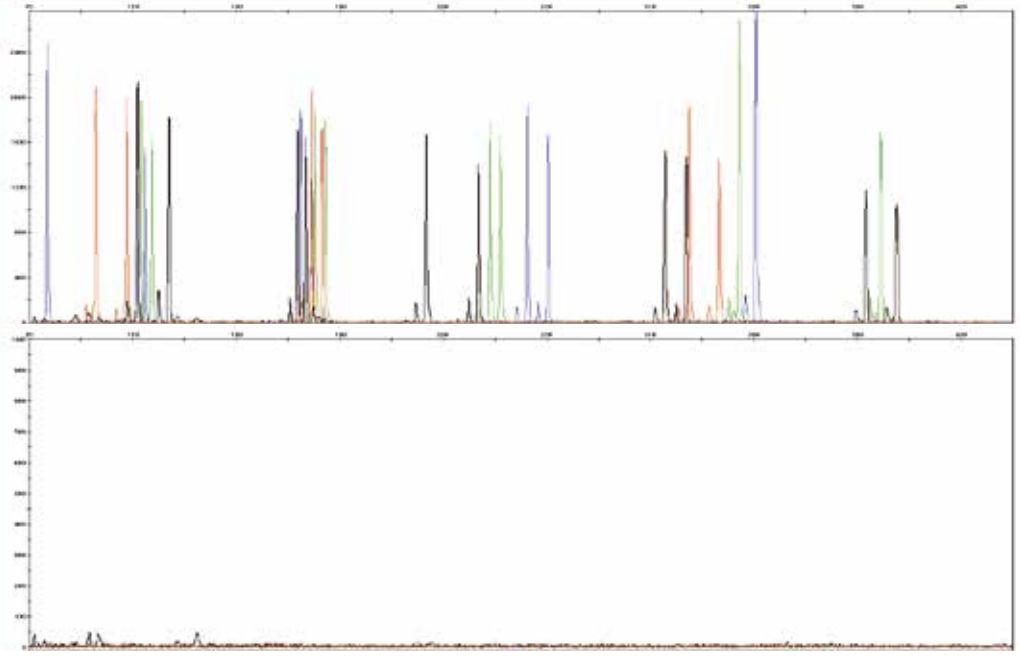

Figure 9. Cross-contamination analysis. Examples of typical sample electropherograms for positive (upper panel) and negative (lower panel) sample punches are shown for the Investigator ESSplex SE GO! Kit.

Conclusions

QIAGEN's Investigator STR GO! chemistry has been developed to streamline processing of reference samples, with a fast reaction speed of only 45 minutes. However, PCR setup for the large numbers of samples typically submitted to DNA database laboratories results in a bottleneck at PCR setup, particularly with paper-based samples, which require punching prior to PCR setup.

Data presented here for both Investigator IDplex GO! and Investigator ESSplex SE GO! Kits on the Hamilton easyPunch STARlet demonstrates a high-throughput, automated solution that removes this bottleneck and enables large numbers (e.g., hundreds) of reference samples to be processed in a single day using a single workflow. Furthermore, manual intervention with this workflow is minimal, ensuring maximum sample and process integrity and freeing up analysts to focus on more challenging samples.

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.

Find out more at **www.qiagen.com/InvestigatorIDKits**.

Ordering **www.qiagen.com/contact** | Technical Support **support.qiagen.com** | Website **www.qiagen.com**

1096441 09/2015