Developmental Validation of STAR Q Punch AS and STAR Q Swab AS Instruments for PCR Setup of Investigator® STR GO! Kits

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Introduction

Worldwide, criminal justice systems are making increasing use of DNA databases to maximize the impact of DNA profiling in human identification. This has markedly increased the number of submissions to these databases and in turn, the need for high-throughput, automated solutions for processing such large numbers of samples.

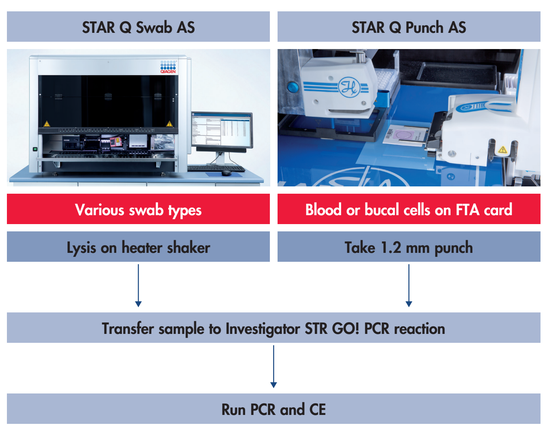
To address this requirement, QIAGEN has developed automated workflows for reference samples collected on cards (e.g., FTA®) and buccal swabs. These two workflows utilize QIAGEN's Investigator STR GO! Kits and automate sample punching/pre-treatment, as well as PCR setup. The workflows use two new QIAGEN® instruments: the STAR Q Punch AS for EasiCollect™ and NUCLEIC-CARD™ samples and the STAR Q Swab AS for various types of buccal swabs.

Here, we describe the developmental validation of these two high throughput workflows and present data using the Investigator 24plex GO! Kit demonstrating reproducible, high-quality DNA profiles consistent with standards expected of manual processing. Furthermore, the Investigator 24plex QS Kits come with an integrated quality control feature – the unique Quality Sensor – which allows the generation of additional, valuable data for performance checks, without affecting PCR performance. The Quality Sensor is able to confirm a successful PCR amplification, and to distinguish between the absence of DNA due to improper sampling from a failed PCR amplification. This information can be used to choose the most appropriate rework strategy and streamline the overall workflow for direct amplification with higher first success rates.

This data establishes the STAR Q workflows and Investigator STR GO! Kits as effective solutions for laboratories looking to increase throughput for reference and database samples.

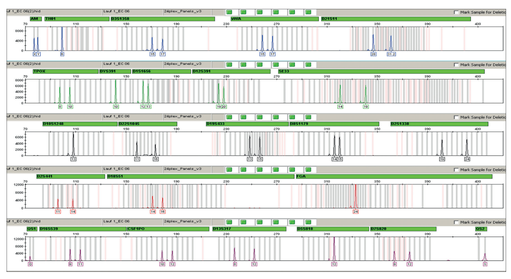
STAR Q AS Instruments

- Up to 384 samples per day with one operator – from sample to CE
- Fully automated FTA card handling, identification of best sample area for punching and PCR setup
- Integrated swab lysis and PCR setup
- Pre-configured and validated protocols for Investigator STR GO! Kits
- Full sample traceability and LIMS compatibility



STAR Q Punch AS and STAR Q Swab AS instrument workflows.

Developmental Validation of the STAR Q Punch AS



Example electropherogram. An EasiCollect sample amplified with the Investigator 24plex GO! Kit is shown.

Protocols were developed for fully automated punching of FTA cards and reaction setup for amplification with QIAGEN Investigator STR GO! Kits on the STAR Q Punch AS instrument. The maximum capacity is 180 cards per run and single or duplicated PCR setups can be performed.

For 96 samples in single setup, a run takes ~90 minutes. Protocols allow the processing of buccal cells on indicating FTA paper (EasiCollect or NUCLEIC-CARD) or blood on FTA paper.

The imaging settings used to identify the best sample area to take a punch are optimized for the individual card and sample type. One punch of 1.2 mm size is punched from the center of the sampling area into a well of a 96-well PCR plate after the PCR reagent master mix has been distributed. The protocol is set to take three punches from a separate card in between sample cards to clean and prevent sample carryover. Data was analyzed at a threshold of 200 RFU using GeneMapper® ID/X v1.2.

Summary of the validation results. Investigator 24plex GO! Kit was used.

Card type	Sample type	Cycle No.	Sample No.	Donor No.	First pass rate
GE Healthcare EasiCollect	Buccal	27	132	22	96%
Copan NUCLEIC-CARDS	Buccal	27	132	22	98%
GE Healthcare FTA card	Blood	25	180	36	100%

Developmental Validation of the STAR Q Swab AS

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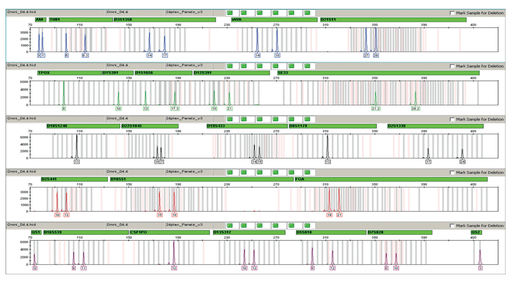

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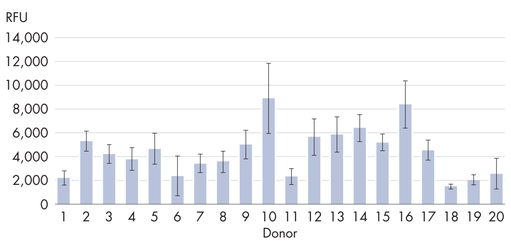

Validation of OmniSwabs. Samples were amplified using the Investigator 24plex GO! Kit Ⓐ Representative electropherogram. Ⓑ Average STR profile signal heights; variation reflects expected donor-dependent differences in shed buccal cells.

STAR Q Swab AS protocols were developed to fully automate swab pre-treatment and STR assay plate setup. Up to two 96 deep-well plates can be loaded in one run. Lysis and assay setup takes ~1 h 45 min. The following swab types were tested:

- OmniSwabs (GE Healthcare)
- Polyester swabs (Puritan)
- Cotton swabs (Sarstedt)
- Flocked swabs (Copan)

Swabs are lysed using 500 µl Investigator STR GO! Lysis Buffer in a 96 deep-well plate on the heated shaker of the instrument. After lysis, 900 µl water is added to each sample to ensure a liquid level well above the swab for the subsequent pipetting step. 2 µl of swab lysate is transferred to the PCR reaction. Amplification was performed on an Applied Biosystems™ GeneAmp™ PCR System 9700 instrument using 27 cycles.

For each swab type tested, 4 samples were collected from 20 different donors (80 swabs in total). Samples were run in checkerboards alternating with empty wells to test for cross-contamination. Full profiles were obtained for all samples in this study at a threshold of 200 RFU. No sample carryover was observed.

Data Interpretation Using the Quality Sensor

Direct amplification of database samples is capable of providing very high first-pass success rates. However, issues in sample collection or storage can cause failures that require rework. Typical failure reasons are sample degradation due to microbial growth promoted under moist storage conditions, and inhibition – for example caused by food ingredients transferred to a buccal swab. In these cases, the Quality Sensor built into the Investigator 24plex GO! Kit provides valuable information to distinguish between degradation and inhibition and define rework accordingly.

- Inhibition swabs: Dilute the swab lysate, re-run PCR.
- Degradation swabs: Process back-up swab if available, or re-run PCR with increased cycle number.
- Inhibition FTA: Take another punch, wash punch or extract DNA if the issue persists.
- Degradation FTA: Take another punch, re-run PCR with increased cycle number.

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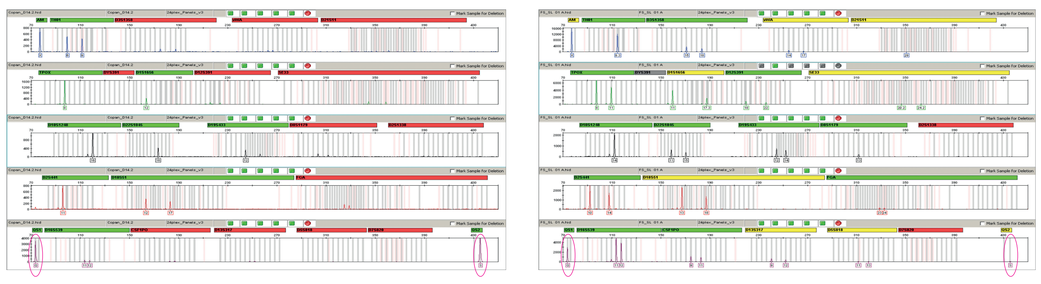

Examples for compromised swab samples. Ⓐ A Sarstedt buccal swab was stored under moist conditions. The presence of both Quality Sensor fragments excludes inhibition, thereby proving degradation as reason for the failure. Ⓑ A Copan flocked swab shows inhibition as indicated by dropdown of the large Quality Sensor fragment.

Conclusions

Data presented here for the Investigator 24plex GO! Kit on the STAR Q Punch and STRA Q Swab AS instruments demonstrate that these high-throughput, automated solutions provide the high success rates and reproducibility expected from reference samples with minimal manual intervention.

- The STAR Q Swab AS and STAR Q Punch AS instruments are QIAGEN's latest automation solution for high-throughput PCR setup for reference samples.
- Pre-validated protocols save time and money, enabling fast implementation and validation.
- High first-pass success rates for FTA cards or buccal swabs.
- The Quality Sensor of Investigator 24plex GO! Kit provides advanced data interpretation tool

Improved performance is achieved whilst maintaining the stringent quality standards expected from QIAGEN Investigator solutions, such as the prevention of contamination. Furthermore, because the protocols are pre-defined and validated, implementation of the STAR Q instruments is faster and easier than on other liquid-handling platforms.

The STAR Q Swab AS and STAR Q Punch AS complete QIAGEN's HID workflow portfolio: whatever your lab's throughput or sample type, we have a solution to streamline your workflow!

The applications presented here are for molecular biology applications. They are not intended for the diagnosis, prevention or treatment of a disease. 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.

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Sample to Insight