

Application Note

The STAR Q Swab AS Instrument for high-throughput PCR setup of QIAGEN's Investigator® STR GO! Kits

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Figure 1. The STAR Q Swab AS instrument.

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 numbers 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 an automated workflow for reference samples collected on buccal swabs. This workflow utilizes QIAGEN's OmniSwab with Investigator STR GO! Kits and automates sample pre-treatment as well as PCR setup using QIAGEN's STAR Q Swab AS Instrument. The workflow can also be used on swabs from other providers to enable maximum flexibility and ease of adoption. Here we describe the developmental validation of the STAR Q Swab AS Instrument and present data using the OmniSwab and Investigator 24plex GO! Kit demonstrating reproducible, high-quality DNA profiles consistent with the standard expected of manual processing but without the variability inherent in manual processing. Furthermore, the Investigator 24plex GO! Kit comes 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. It is able to confirm a successful PCR amplification and to distinguish between the absence of DNA due to improper sampling and a failed PCR amplification, as well as differentiating between degradation and inhibition. 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 OmniSwab and STAR Q workflow with Investigator STR GO! Kits as an effective and easily adopted solution for laboratories looking to automate their manual process or increase throughput for reference and database samples. Furthermore, data generated with alternative swabs from other providers demonstrates the applicability of the workflow for laboratories using other swabs but wishing to benefit from the efficiency of the STAR Q Swab solution.

Methods

Swab testing

STAR Q Swab AS protocol scripts were developed to fully automate both swab pre-treatment and STR assay plate setup. QIAGEN OmniSwabs, Sarstedt® cotton swabs, Puritan® polyester swabs and Copan® flocked swabs were all tested. Swab pre-treatment is carried out on the STAR Q Swab AS instrument itself, involving 30 minutes heated lysis in a heater/shaker at 750 rpm using 500 µl of QIAGEN's Investigator STR GO! Lysis Buffer, followed by cooling in a cooling device on the instrument deck. Sterile distilled water (900 µl) was then added to each sample to ensure a liquid level well above the swab for the subsequent pipetting step. For each sample, 20 µl of PCR master mix was prepared from the Investigator 24plex GO! Kit reagents and dispensed into a 96-well PCR plate using the STAR Q Swab AS pipetting channels and disposable tips. A 2 µl aliquot of the sample lysate was then removed from each sample and added to the PCR reaction mix. Assay plates were amplified using an Applied Biosystems® GeneAmp® PCR System 9700 thermal cycler. PCR was performed according to the corresponding kit handbooks, using 27 PCR cycles. Samples were run on an Applied Biosystems 3500 Genetic Analyzer and analyzed with Applied Biosystems GeneMapper® ID-X Software v1.2 using a 200 RFU allele-calling threshold.

Cross-contamination checkerboard testing

A cross-contamination analysis was carried out to test for potential sample carryover. Buccal swab samples were arranged in checkerboard patterns in the sample input plate, alternating wells containing a swab with empty wells. Samples were taken from different donors to allow tracing of potential sample carryover. Samples were amplified using the Investigator 24plex GO! Kit for 27 PCR cycles. Data were analyzed with the Applied Biosystems GeneMapper ID-X Software v1.2 using a threshold of 200 RFU for detection of alleles in empty wells.

Results

Swab testing

Full DNA profiles were obtained for all swab samples. An example STR profile is shown in Figure 2.

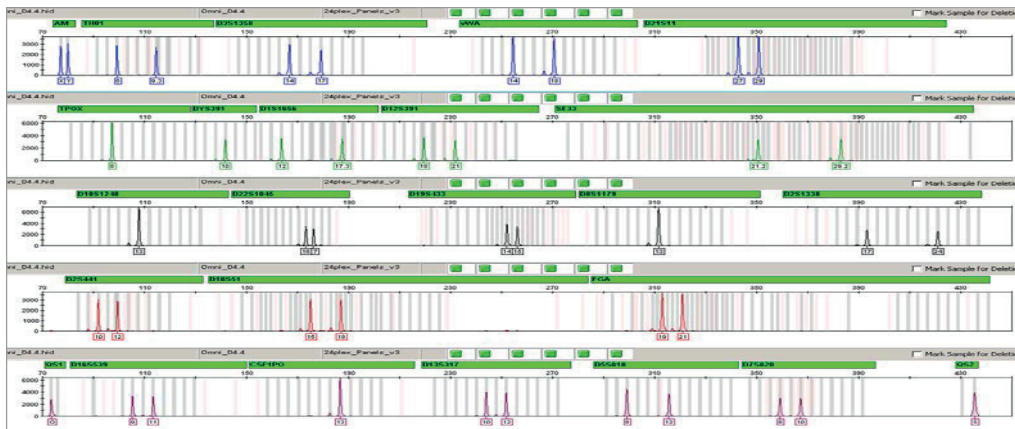


Figure 2. Example STR profile for a QIAGEN OmniSwab amplified with the Investigator 24plexGO! Kit. Data analysis was performed using Applied Biosystems GeneMapper ID-X Software.

Peak heights were evaluated for all swab types, with 20 donors and 4 replicates of each donor being analyzed. Peak height data is shown in Figure 3.

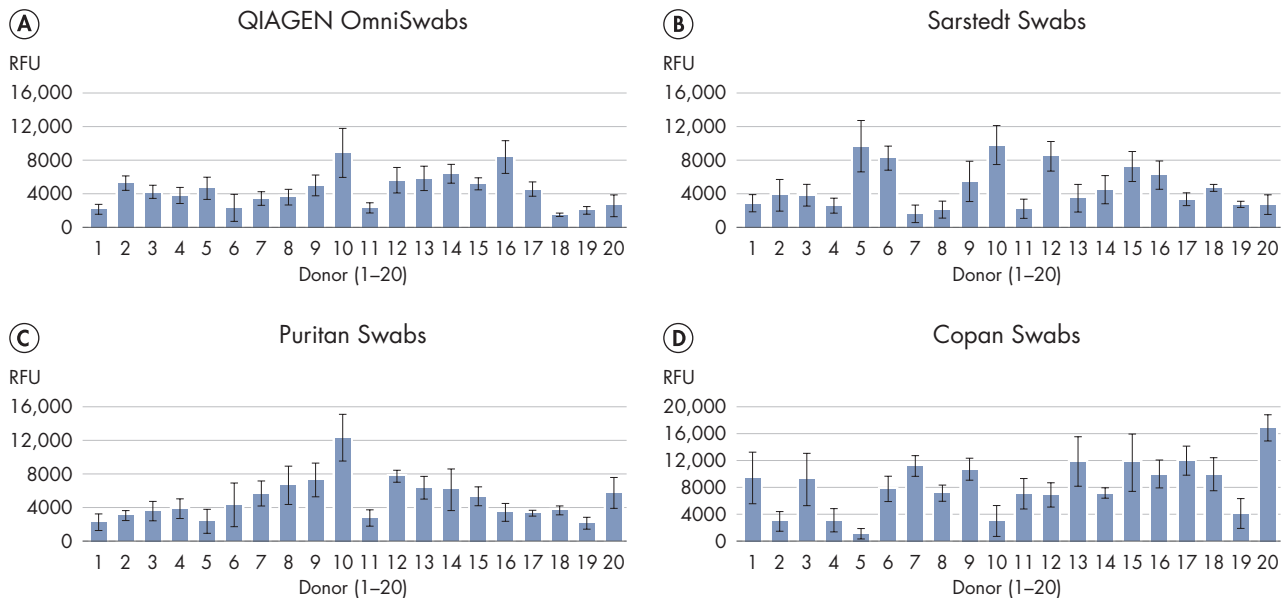


Figure 3. Analysis of peak height variation from buccal swabs. Swabs were collected on **A** QIAGEN OmniSwabs, **B** Sarstedt swabs, **C** Puritan swabs and **D** Copan swabs. Average peak heights for 20 different donors processed in 4 replicates are shown for each swab type. Samples were amplified and analyzed using the QIAGEN® Investigator 24plex GO! Kit, according to manufacturer's instructions.

Cross-contamination checkerboard testing

Checkerboards evaluating the risk of cross-contamination demonstrated 100% full profiles at a 200 RFU threshold, with no evidence of allelic carry-over to water samples.

Conclusion

QIAGEN's Investigator STR GO! Kits for direct amplification are designed to enable streamlined processing of reference samples. The development of an automated solution for Investigator STR GO! chemistry on the STAR Q Swab AS instrument provides a high-throughput workflow for this chemistry, well suited for labs receiving large numbers of buccal swabs.

The data presented here for the OmniSwab and Investigator 24plex GO! Kit on the STAR Q Swab AS demonstrate that this high-throughput, automated solution provides the high success rates and reproducibility expected from reference samples with minimal manual intervention. This performance is also achieved while maintaining the stringent quality standards expected from QIAGEN Investigator solutions, such as prevention of contamination. Furthermore, because the protocols are pre-defined and validated, implementation of the STAR Q Swab AS is faster and easier than on other liquid handling platforms.

Ordering Information

Product	Contents	Cat. no.
STAR Q Swab AS Instrument	Includes installation and training, 1-year warranty on parts and labor and 1 year maintenance	9002652
Investigator 24plex GO! Kit (200)	Primer Mix, Fast Reaction Mix 2.0 including <i>Taq</i> DNA polymerase, Control DNA, allelic ladder 24plex, DNA size standard	382426
Investigator 24plex GO! Kit (1000)	Primer Mix, Fast Reaction Mix 2.0 including <i>Taq</i> DNA polymerase, Control DNA, allelic ladder 24plex, DNA size standard	382428
Investigator STR GO! Lysis Buffer (200)	Lysis buffer for 200 swab samples	386516
OmniSwab, Sterile (100)	Sterile collection swabs with ejectable brush-like head used for 100 buccal or saliva samples	WB100035

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