

# High-throughput Investigator<sup>®</sup> 24plex GO! PCR setup from EasiCollect<sup>™</sup> Plus cards using the STAR Q Punch AS Instrument

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## Introduction

The increasing utilization of DNA databases as an investigative tool in criminal investigations has stimulated a marked increase in arrestee and suspect sample submissions for DNA profiling. However, this growth in the collection and processing of database samples brings new challenges. Improper sample collection, storage and transport can result in a considerable number of submissions arriving at the database laboratory being deemed unsuitable for processing. Moreover, the analysis of large numbers of samples in the laboratory requires significant resource allocation if quality and operational failures are to be avoided. Even with the most optimized processes, some samples may fail to produce reliable results, and laboratories have to spend additional resources for determining the underlying causes of failure and planning an efficient rework strategy.

## Increasing throughput while maintaining quality

Here we present QIAGEN's complete high-throughput workflow solution that addresses these challenges while ensuring the highest quality standards and first-pass success rates. A total of 360 samples can be processed in a single day by a single operator using this workflow.

### Sample collection

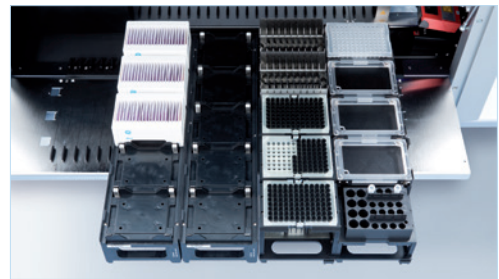
Sample collection is simplified with the introduction of the EasiCollect Plus. This device is simple to use and includes a framed Indicating FTA Card that facilitates reproducible collection of high-quality DNA in a format suitable for room temperature storage and transportation.



**Figure 1.** EasiCollect Plus sample collection device with framed Indicating FTA Card.

### Sample punching and reaction setup

Once transferred to the laboratory, sample punching (where a small section of the collection card is punched out for DNA profiling) is automated on the STAR Q Punch AS, which also enables PCR reaction setup for the Investigator 24plex GO! Kit and maintains a record of every sample processed.



**Figures 2 and 3.** The STAR Q Punch AS Instrument.

### PCR and process feedback

The Investigator 24plex GO! Kit is an expanded CODIS format PCR kit which includes a unique Quality Sensor. The Quality Sensor acts as an internal performance control for the final PCR reaction, enabling analysts to identify the reason for a failed amplification and ensuring that resources are not wasted in processing samples of poor quality.



**Figure 4.** The Investigator 24plex GO! Kit.

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## Materials and methods

### Samples

Forty-four buccal samples were collected using the EasiCollect Plus sample collection device. These samples were stored at room temperature before processing.

### Instrument setup

EasiCollect Cards were removed from each EasiCollect Plus device and loaded onto the EasiCollect Plus 20-Slot Rack prior to being loaded onto the STAR Q Punch AS. A total of 180 cards can be loaded onto the STAR Q Punch AS in a single run.

### PCR setup

The STAR Q Punch AS includes a pre-loaded protocol for the Investigator 24plex GO! Kit. This was used to set up 22  $\mu$ l PCR reactions in 96-well PCR plates, according to the manufacturer's instructions. This volume includes 2  $\mu$ l of the Investigator STR GO! Punch Buffer which is used to overcome the influence of potential PCR inhibitors present on the FTA paper.

### Card punching

Using a pre-loaded protocol designed for EasiCollect Plus Cards, the STAR Q Punch AS individually selected each sample card and by utilizing a built-in imaging software to identify the appropriate area for punching, it punched a 1.2 mm section of the card into the 96-well PCR plate containing the Investigator 24plex GO! PCR reaction. For each sample punch, the instrument collected a picture of the appropriate PCR well before and after punching. This ensures that punching into the PCR well in question is successful. To ensure that there was no carry-over of DNA/cellular material between samples, three clean punches were taken from separate, empty/fresh cards before punching of the next sample.

### PCR, CE and analysis

PCR plates were removed from the instrument and placed on a 9700 thermal cycler before undergoing PCR amplification, according to the manufacturer's instructions. Capillary electrophoresis was carried out on the 3500 Genetic Analyzer with injection settings of 1.2 kV and 30 s, prior to analysis using the GeneMapper® ID-X. Samples were analyzed with an analytical threshold of 100 RFU.

### Cross-contamination study

The same 44 samples were analyzed in a cross-contamination study, whereby samples were prepared in a 96-well plate in between PCR reagent blanks using a checkerboard pattern (Figure 5).

No.	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Negative	Sample 8	Negative	Sample 16	Negative	Sample 23	Negative	Sample 31	Negative	Sample 38	Negative
B	Negative	Sample 5	Negative	Sample 12	Negative	Sample 20	Negative	Sample 27	Negative	Sample 35	Negative	Sample 42
C	Sample 2	Negative	Sample 9	Negative	Sample 17	Negative	Sample 24	Negative	Sample 32	Negative	Sample 39	Negative
D	Negative	Sample 6	Negative	Sample 13	Negative	Sample 21	Negative	Sample 28	Negative	Sample 36	Negative	Sample 43
E	Sample 3	Negative	Sample 10	Negative	Sample 18	Negative	Sample 25	Negative	Sample 33	Negative	Sample 40	Negative
F	Negative	Sample 7	Negative	Sample 14	Negative	Sample 22	Negative	Sample 29	Negative	Sample 37	Negative	Sample 44
G	Sample 4	Negative	Sample 11	Negative	Sample 19	Negative	Sample 26	Negative	Sample 34	Negative	Sample 41	PC
H	Negative	Ladder	Negative	Sample 15	Negative	Ladder	Negative	Sample 30	Negative	Ladder	Negative	NTC

Figure 5. Checkerboard cross-contamination study.

## Results

### Performance testing

All 44 samples gave full, balanced DNA profiles (Figure 6). A significant variation in relative peak heights between the samples was observed, which is typical of buccal samples, and can be heavily influenced by the thoroughness with which the sample is collected by an individual and the availability of DNA from the subject.

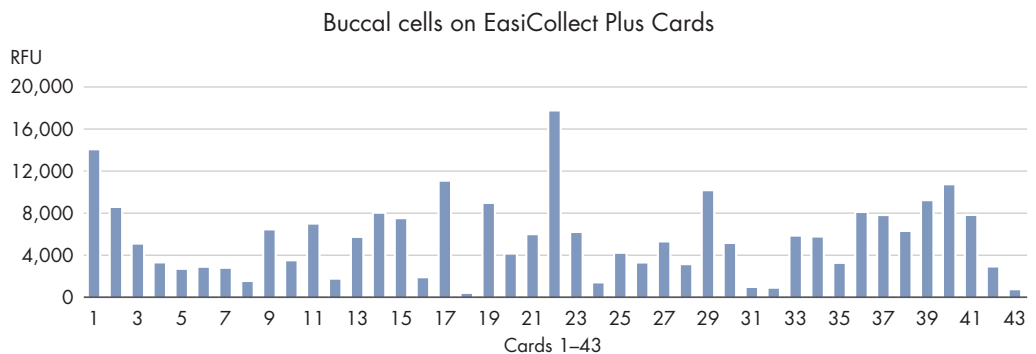
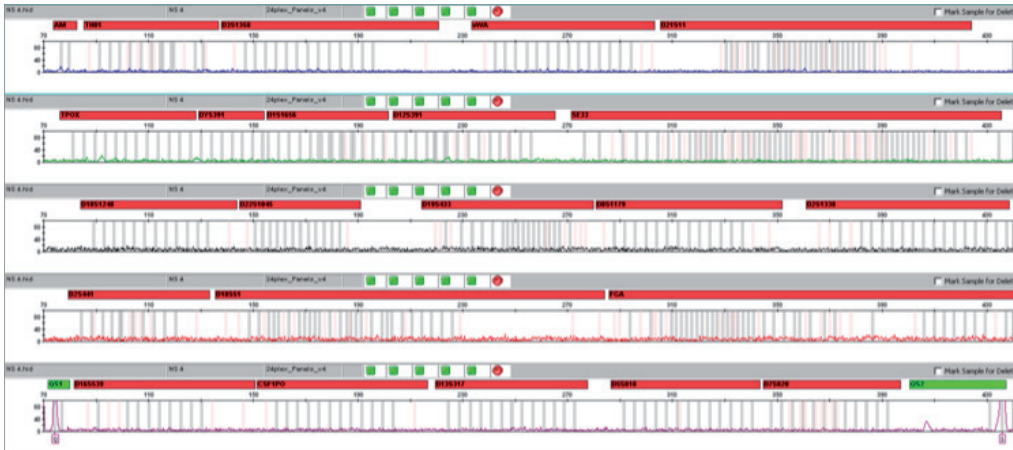


Figure 6. Relative peak heights of all 44 samples processed. All samples gave full, balanced profiles.

### Cross-contamination study

All 44 positive samples gave full profiles, with no evidence of allelic peaks above the threshold in any of the negative sample wells. The Quality Sensor present in the Investigator 24plex GO! Kit enabled verification of a successful PCR reaction in every negative sample well (Figure 7). In a database environment, this feature would enable the analyst to identify samples that failed due to a lack of amplifiable DNA and prevent futile reworking of such samples.



**Figure 7. A typical electropherogram from a negative sample processed in the checkerboard cross-contamination study. The Quality Sensor peaks at either end of the purple channel, indicating a successful PCR.**

## Conclusion

The comprehensive workflow presented here addresses the unique challenges facing DNA database labs when receiving and processing a large number of samples every day. Sample collection is standardized with the EasiCollect Plus sample collection device, which also allows for simple and inexpensive storage and transport at room temperature. Assay setup is automated on the STAR Q Punch AS, enabling up to 360 samples to be prepared for PCR each day, by a single operator. The Investigator 24plex GO! Kit ensures high success rates for database samples, and an integrated Quality Sensor helps identify the cause of a failed PCR run and allows you to focus your resources on the most promising of your DNA samples. Furthermore, with a full workflow solution from a single provider, implementation and support responsibilities are clear and give full peace of mind.

## Summary

- Complete QIAGEN workflow including sample collection, punching, assay setup and STR assay
- Pre-validated protocols for the complete workflow enable fast and simple adoption
- Quality Sensor ensures no futile reworking of poor quality samples

## Ordering Information

Product	Contents	Cat. no.
Investigator 24plex GO! Kit (200)*	Primer Mix, Fast Reaction Mix 2.0 including Taq DNA polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO)	382426
Investigator STR GO! Punch Buffer (200)*	Lysis buffer for 200 samples of epithelial cells on paper	386526
STAR Q Punch AS Instrument	Instrument with 1-year warranty on parts. For use with QIAGEN EasiCollect Cards, Bode Buccal Collectors and Copan NUCLEIcard system collection cards	9002651
EasiCollect (50)	50 x buccal sample collection devices with built-in foam applicator and framed Indicating FTA card (EasiCollect Card)	WB120462
EasiCollect Plus 20-Slot Rack (25)	25 x reusable sample card racks for 20 EasiCollect Cards for use as carrier on the deck of a STAR Q Punch AS instrument	WB120240

\* Other sizes available; see [www.qiagen.com](http://www.qiagen.com).

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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

Trademarks: QIAGEN®, Sample to Insight®, Investigator® EasiCollect™, FTA™ (QIAGEN Group), GeneMapper® (Thermo Fisher Scientific).  
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