Application Note

Direct amplification and an accelerated workflow in skin flake isolation with the Investigator® Casework GO! Kit

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Introduction

Forensic casework trace samples require efficient DNA recovery to enable successful downstream analysis. This is particularly true for samples containing mixtures of DNA where results can be ambiguous or inconclusive. Skin flake isolation, although laborious, provides a solution to avoid mixture deconvolution during DNA analysis and interpretation. Consequently, these samples become more valuable due to the increased effort to prepare. Therefore, maximizing DNA yield is imperative to ensure downstream success. The Investigator Casework GO! Kit provides a solution to increase DNA yield while shortening and simplifying the laboratory process.

The Investigator Casework GO! Kit

The Investigator Casework GO! Kit can be used for the pretreatment of forensic samples in a direct amplification workflow. The kit permits sample lysis without DNA purification and the lysate is compatible with several downstream options including the Investigator Quantiplex® Pro Kit and Quantiplex PRO RGQ Kit for sample quantification or the Investigator 24plex QS, ESSplex SE QS or IDplex Plus kits for STR amplification. The Investigator Casework GO! Kit can be used for direct amplification of casework samples and thus enables an accelerated workflow in casework analysis without the need for further purification. This can enable laboratories to increase sample throughput bringing closure to cases sooner.

Skin flake analysis and isolation

Dander isolation provides a powerful tool to separate individual skin flakes for analysis to avoid complex DNA profile deconvolution. However, hundreds of skin flakes must be collected and tested as historically only 10–20% of particles contain enough DNA for downstream analysis. Skin flakes are isolated under stereoscopic magnification (30–50 fold) using micro tweezers and sent for downstream processing. Traditionally, an overnight Chelex® extraction was performed. Implementing the Investigator Casework GO! Kit can improve DNA yield while simultaneously simplifying the workflow. ▷

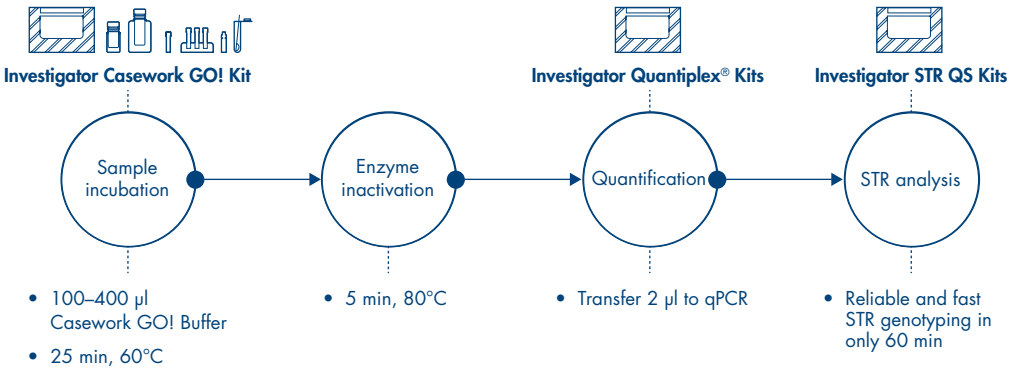

Figure 1. The Investigator Casework GO! Kit workflow.

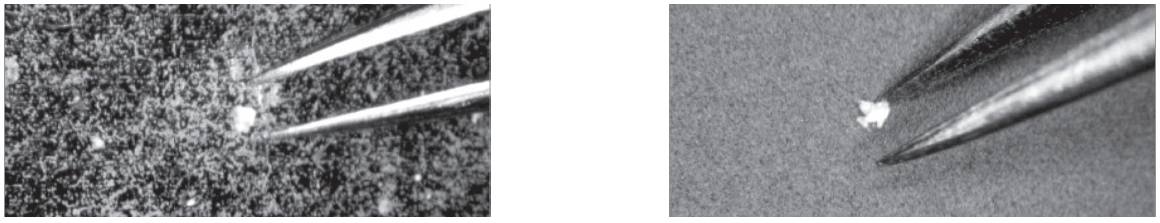

Figure 2. Skin flake isolation under stereoscopic magnification (used by permission of Dr. Harald Schneider).

Methods

Three DNA extraction methods are compared in Table 1: ReadyAmp® Genomic DNA Purification (Chelex), Investigator STR GO! Lysis Buffer and Investigator Casework GO! Buffer. For Chelex extracted samples, sample incubation was a minimum of 4 hours, but this often extended to an overnight lysis. The indicated volume of Investigator Casework GO! Buffer to be added is between 100–400 µl (Figure 1). This was reduced to 38 µl plus 2 µl proteinase K to normalize the three methods and concentrate the lysate.

Table 1. Summary of methods of DNA extraction from skin flakes

	ReadyAmp Genomic DNA Purification Resin (Chelex)	Investigator STR GO! Lysis Buffer	Investigator Casework GO! Buffer
Sample number	996	996	913
Chemistry components and volume	40 µl Chelex + 2 µl Proteinase K + 2.5 µl Tween® 10%	20 µl GO! Lysis Buffer + 20 µl water + 2 µl Proteinase K + 2.5 µl Tween 10%	38 µl Casework GO! Buffer + 2 µl Proteinase K
Incubation time and temperature	4 h (minimum), 56°C	10 min, 95°C	25 min, 60°C; then 5 min, 80°C

Results

The Quantifiler® Duo Real Time PCR assay was used for quantification. Average DNA concentration between the methods (Figure 3) identifies that both the modified Investigator STR GO! Lysis Buffer and Investigator Casework GO! Buffer methods significantly increased sample yield compared with the Chelex procedure. Furthermore, the percentage of dander that can be used for downstream evaluation also increased with both the Investigator STR GO! Lysis Buffer and Investigator Casework GO! Buffer methods compared to the Chelex methodology (Figure 4).

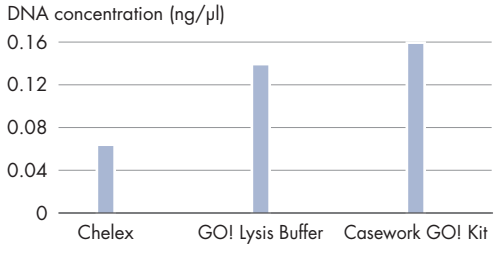

Figure 3. Average DNA concentration (ng/µl) obtained using three methods of extraction: Chelex, Investigator STR GO! Lysis Buffer and Investigator Casework GO! Buffer.

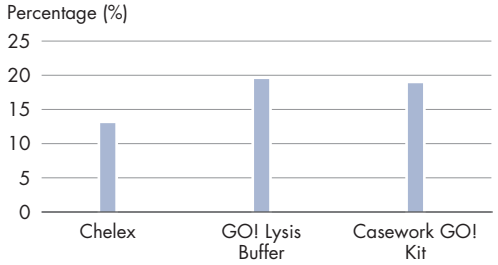

Figure 4. Percentage of dander suitable for downstream evaluation using three methods of extraction: Chelex, Investigator STR GO! Lysis Buffer and Investigator Casework GO! Buffer.

Both the Investigator STR GO! Lysis Buffer and Investigator Casework GO! Kit showed improvements over Chelex in sample yield and number of evaluable dander. The Investigator Casework GO! Kit outperformed the Investigator STR GO! Lysis Buffer when assessing the downstream electropherograms. Using the Investigator ESSplex SE QS Kit for STR typing, observations of sample inhibition and loci drop-out were seen in the higher molecular weight region from lysates using the adapted Investigator STR GO! Lysis Buffer method. Figure 5 is an example of an inhibited sample profile including loci drop-out. However, this was not the case when using the Investigator Casework GO! kit (Figure 6).

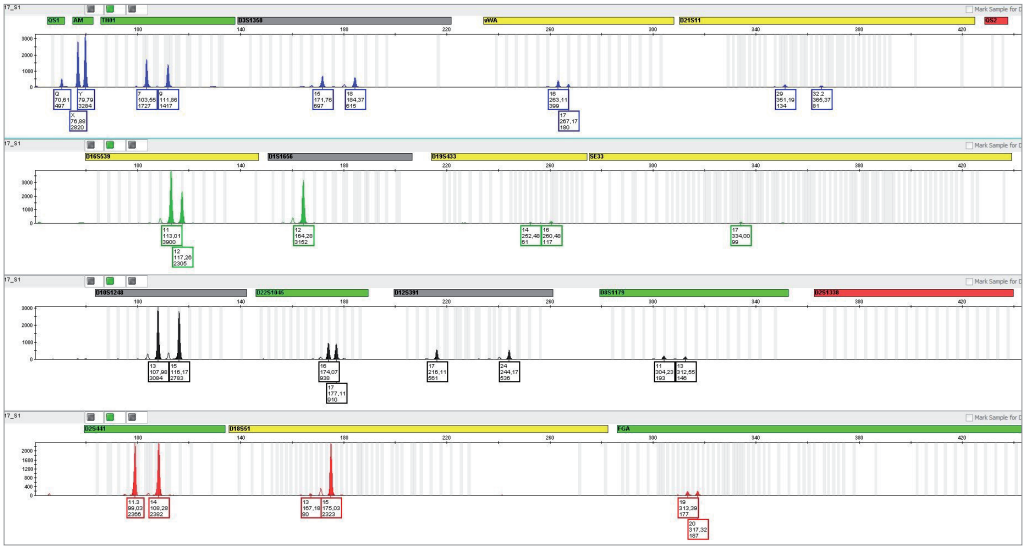

Figure 5. Example electropherogram using the adapted Investigator STR GO! Lysis Buffer method showing an inhibited sample profile including loci drop-out.

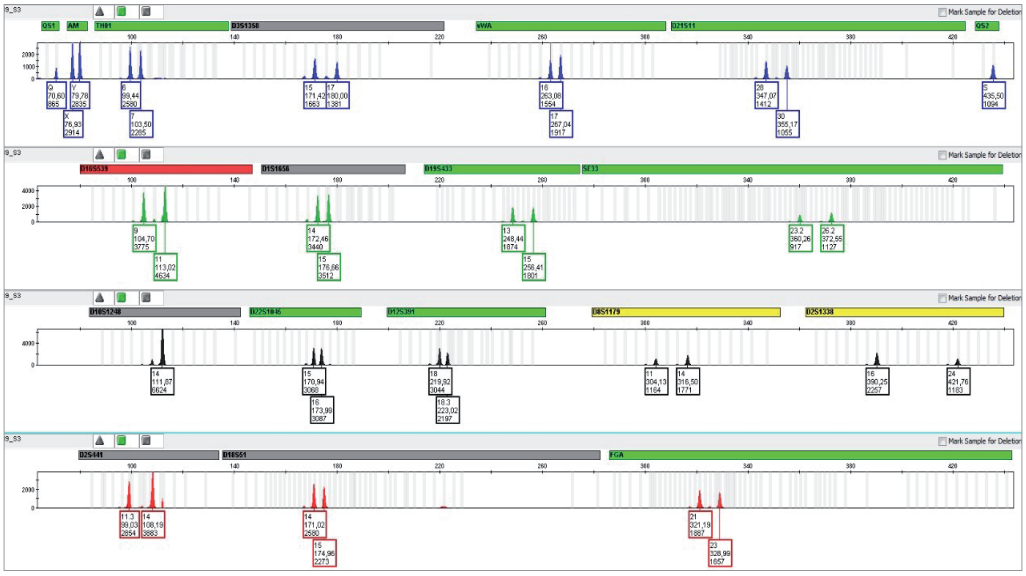

Figure 6. Example electropherogram using the Investigator Casework GO! Kit showing a balanced profile with no signs of inhibition.

The resulting profile from the Investigator Casework GO! Kit lysate provides a complete, balanced profile with no signs of inhibition. This is supported by the presence of both Quality Sensor 1 (QS1) and Quality Sensor 2 (QS2) in relative proportion to each other (Table 2).

Table 2. Quality sensor appearances and their interpretation

Allele Peaks	QS1	QS2	Interpretation
Present	Present	Present	Successful profile
Absent	Present	Present	No DNA
Absent	Absent	Absent	Failed PCR
Ski-slope profile	Present	Dropdown	Inhibitors present
Ski-slope profile	Present	Present	Degraded DNA

Conclusion

Pairing skin flake isolation with the Investigator Casework GO! Kit improved DNA yield comparative to the traditional Chelex extraction methodology. Critically, we also observed an increase in samples suitable for DNA analysis enabling improved success rates and closure to cases. This kit offers improvements in sample yield together with a significant reduction in lysis time from a minimum of 4 hours down to just 30 minutes. This allows an increased sample throughput for the laboratory while simultaneously simplifying the manual workflow by removing the need to separate the lysate.

Summary

With the Investigator Casework GO! Kit (ISO 18385 Forensic Grade):

- Guarantee the quality of your important casework samples
- Maximize throughput and use of your existing resources
- Enjoy easy implementation thanks to established and validated protocols

Ordering Information

Product	Contents	Cat. no.
Investigator Casework GO! Kit	Casework GO! Lysis Buffer, Proteinase K Solution and nuclease-free water	386546
Related Products		
Investigator ESSplex SE QS Kit (400)	Primer Mix, Fast Reaction Mix 2.0 including *Taq* DNA Polymerase, Control DNA, allelic ladder, ESSplex SE QS, DNA size standard 550 (BTO), nuclease-free water	381577
Investigator ESSplex SE QS Kit (100)	Primer Mix, Fast Reaction Mix 2.0 including *Taq* DNA Polymerase, Control DNA, allelic ladder, ESSplex SE QS, DNA size standard 550 (BTO), nuclease-free water	381575
Investigator STR GO! Lysis Buffer (200)	Lysis buffer for 200 swab samples	386516

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.