Application Note

High-throughput sample preparation for forensic casework using the Investigator® STAR Lyse&Prep chemistry on the Tecan® Freedom EVO® automated platform

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Introduction

Modern criminal justice relies increasingly on forensic science to identify suspects and bring about convictions, with DNA profiling in particular established as the method of choice for human identification. As a consequence, submissions to forensic DNA profiling laboratories are mounting, forcing labs to find solutions that maximize throughput while maintaining quality and success rates. Here we demonstrate that high-throughput sample purification can be achieved on the Tecan Freedom EVO automated platform using QIAGEN's Investigator STAR Lyse&Prep Kit. This method results in comparable performance and similar yields of PCR-ready, inhibitor-free DNA as with the QIAsymphony® SP instrument, which would allow forensic casework laboratories tasked with managing ever-increasing workloads to increase their capacity.

The Investigator STAR Lyse&Prep Kit

The Investigator STAR Lyse&Prep Kit is designed for automated purification of total DNA from samples encountered in forensic and human-identity applications. The extraction chemistry uses QIAGEN's proven magnetic-particle technology and provides high-quality DNA free of proteins, nucleases and inhibitors, which is suitable for direct use in downstream applications, including quantitative PCR amplification and STR analyses or for storage for later use.

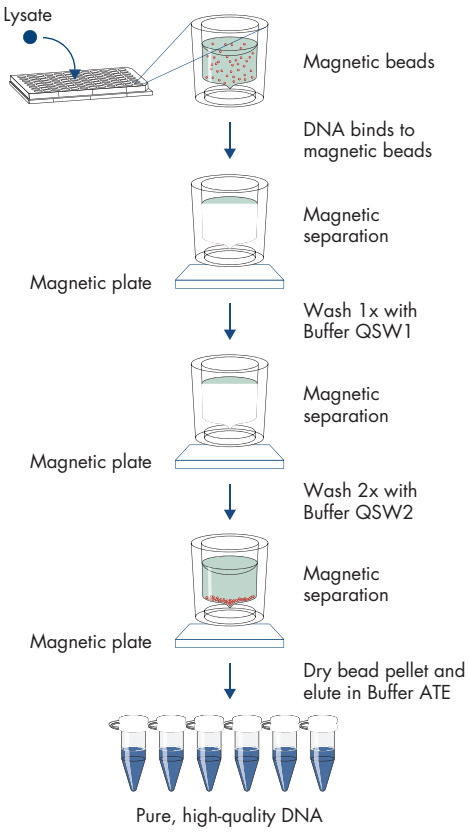

Figure 1. The Investigator STAR Lyse&Prep Kit workflow.

The TECAN Freedom EVO automated platform

The Tecan Freedom EVO automated platform can perform all the steps of the sample extraction procedure after lysis according to pretreatment protocols. Up to 96 samples can be processed in a single run. Extraction protocols for 300 µl and 500 µl sample lysate volumes are available. DNA can be eluted in 30 to 100 µl low-TE buffer.

Methods

Sample linearity and sensitivity

To determine the range of sample input amounts that can be reliably processed using the Investigator STAR Lyse&Prep Kit, serial dilutions consisting of five different concentrations of an individual's blood, saliva and semen samples were used. The samples were extracted and quantified in replicates of five to assess the quality of results for each sample type. The study was conducted using the 300 µl tube-to-plate protocol, and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex® Pro RGQ Kit on a QIAGEN Rotor-Gene® Q Real-Time PCR Cycler. The quantification results were analyzed using the Quantiplex Pro Quant Assay Data Handling Tool v3.3.

DNA yields increased in proportion to the amount of sample extracted (Figures 2–4), and DNA was efficiently recovered from the lowest concentration replicate tested. Observed yields were comparable to those obtained using the QIAsymphony DNA Investigator Kit with corresponding protocols on the QIAsymphony SP.

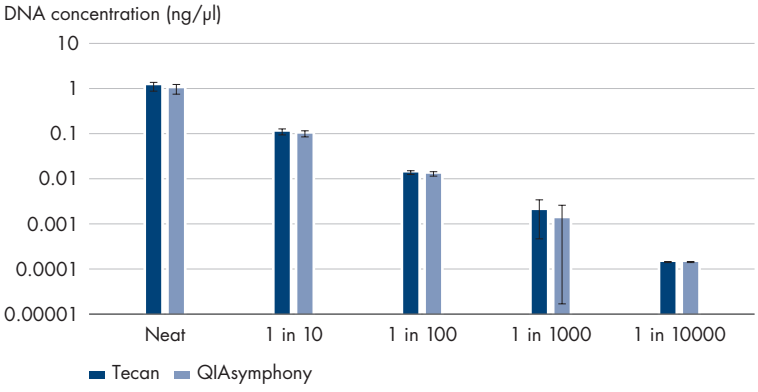

Figure 2. Sensitivity and linearity of DNA extraction from blood using the Investigator STAR Lyse&Prep Kit. Results obtained were comparable between protocols run on Tecan Freedom EVO and QIAsymphony SP instruments.

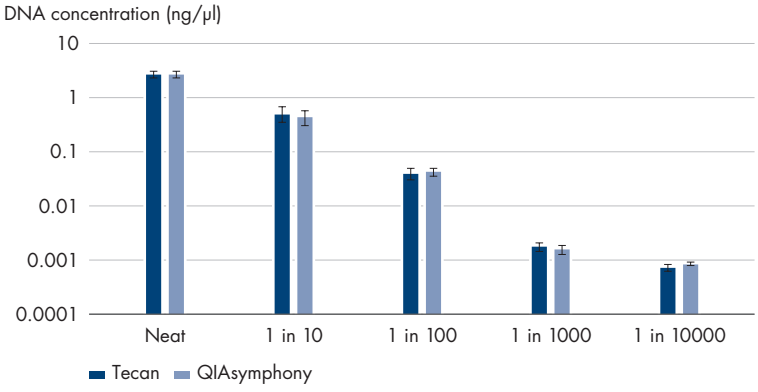

Figure 3. Sensitivity and linearity of DNA extraction from saliva using the Investigator STAR Lyse&Prep Kit. Results obtained were comparable between protocols run on Tecan Freedom EVO and QIAsymphony SP instruments.

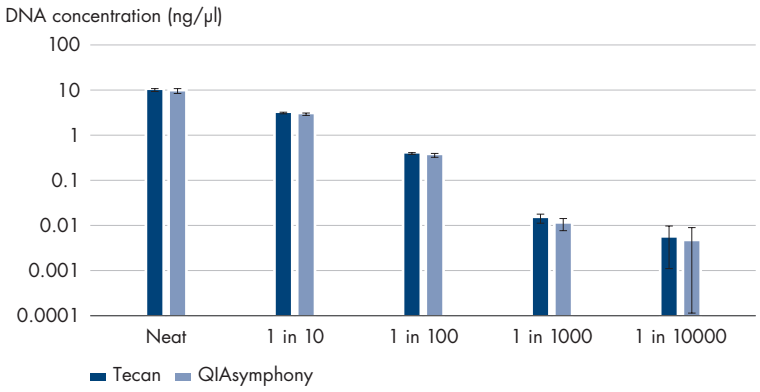

Figure 4. Sensitivity and linearity of DNA extraction from semen using the Investigator STAR Lyse&Prep Kit. Results obtained were comparable between protocols run on Tecan Freedom EVO and QIAsymphony SP instruments.

Removal of PCR inhibitors

Forensic casework samples are frequently contaminated with potential inhibitors of PCR. These inhibitors must be removed during extraction to prevent negative impacts on the final analyses.

The Investigator STAR Lyse&Prep Kit was tested on samples spiked with known inhibitors. Humic acid (1000 ng/µl) and hematin (3500 µM) were added to saliva samples. The study was conducted using the 300 µl protocol, and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex Pro RGQ Kit on a Rotor-Gene Q, and 500 pg of template DNA per reaction was used for Investigator 24plex QS STR PCR reactions. No change in amplification of the Investigator Quantiplex Pro RGQ Kit internal control was observed for any of the samples, which indicates that no inhibition was present (Figure 5). Furthermore, all samples provided full STR profiles without any indication of inhibition. These findings were supported by balanced amplification of the Investigator 24plex Quality Sensor (data not shown).

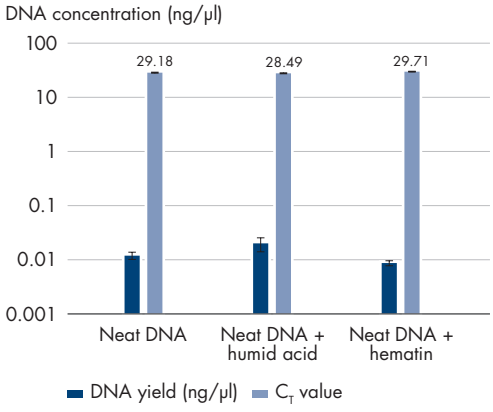

Figure 5. DNA extraction and amplification from samples with added contaminants using the Investigator STAR Lyse&Prep Kit. Humic acid (1000 ng/µl) and hematin (3500 µM) were spiked into neat saliva. Five replicates were processed. DNA yield (dark blue bars, ng/µl) and the C_T values (light blue bars) of the internal control of the Investigator Quantiplex Pro RGQ Kit are shown.

Repeatability and reproducibility

To test the repeatability and reproducibility of the Investigator STAR Lyse&Prep Kit, DNA recovery was determined for 8 runs performed over different days (Figure 6). For each run, 8 samples were used, for a total of 64 samples. The study was conducted using the 300 µl protocol, and DNA was eluted in 50 µl. Samples were quantified using the Investigator Quantiplex Pro RGQ Kit on a Rotor-Gene Q.

PCR amplification was performed using an Applied Biosystems® GeneAmp® 9700 thermal cycler according to the manufacturer's instructions. Capillary electrophoresis was carried out on the Applied Biosystems 3500 genetic analyzer with injection settings of 1.2kV/30 sec before analysis using GeneMapper IDX with an analytical threshold of 100 RFU.

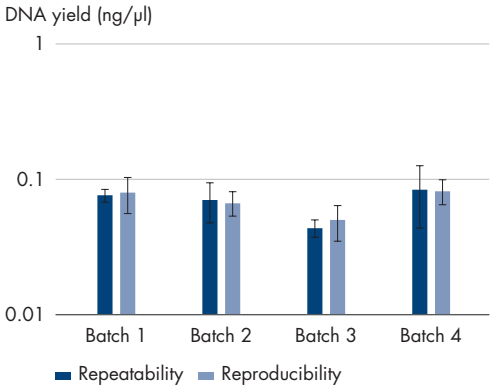

Figure 6. Repeatability and reproducibility of DNA extractions using the Investigator STAR Lyse&Prep Kit. Results were comparable between and within groups, demonstrating reliability of the Investigator STAR Lyse&Prep chemistry to consistently provide high-quality DNA. Eight samples were processed in 8 replicates for a total of 64 samples.

Cross contamination and plate homogeneity

A plate-homogeneity and contamination study was performed using the Investigator STAR Lyse&Prep Kit 300 µl protocol and saliva (neat) arranged in a checkerboard pattern with alternating negative extraction controls. The average concentration of the neat saliva eluates was 4.7 ng/µl (Figure 7). ▷

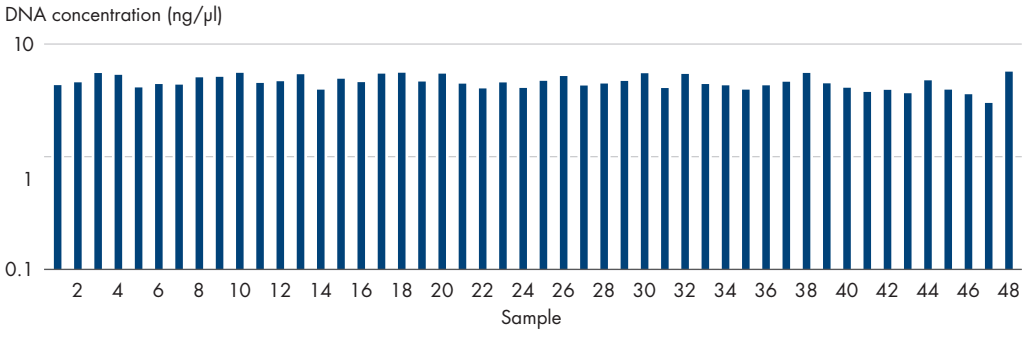

Figure 7. Homogenous extraction of DNA across a 96-well plate using the Investigator STAR Lyse&Prep Kit. Consistent DNA yields were obtained for each sample across a 96-well plate, indicating plate homogeneity and no bias for any particular plate position.

Negative samples were amplified using the Investigator 24plex QS Kit using 15 µl as template. Analysis was repeated for samples showing spurious peaks above 50 RFU; no spurious peak could be verified (Figure 8).

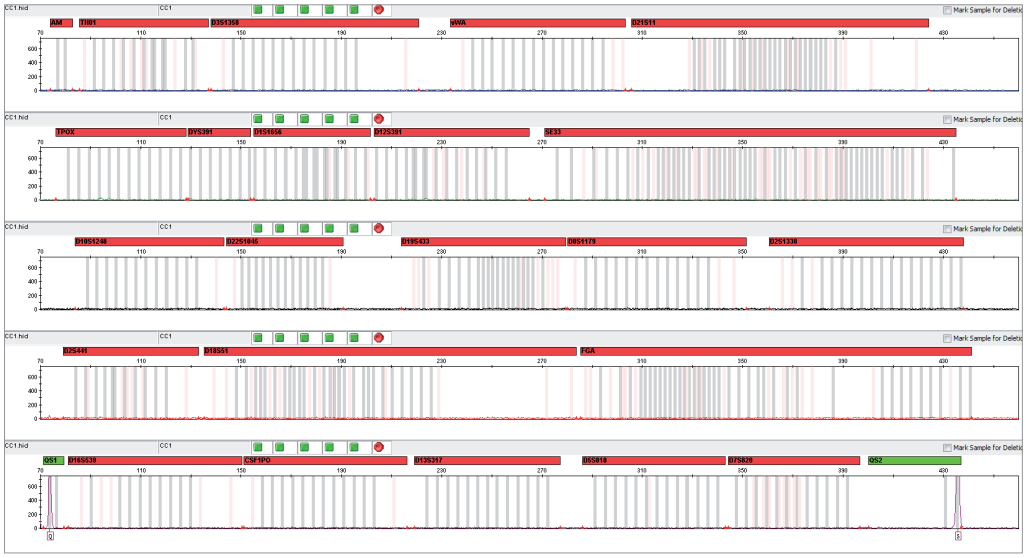

Figure 8. Example electropherogram for a negative sample. There was no amplification of any allelic peaks, but the presence of the quality sensors (far left and far right in the bottom channel) indicate that the PCR itself was successful.

Conclusion

The Investigator STAR Lyse&Prep Kit is a forensic-grade DNA purification kit designed for recovery of high yields of PCR-ready DNA from challenging casework samples. The kit format is specially designed for compatibility with liquid handlers such as the Tecan Freedom EVO automated platform, enabling high-throughput processing of casework samples with no compromise on quality or on success rates. The workflow presented here (Figure 1) using the Investigator STAR Lyse&Prep Kit in combination with the Tecan Freedom EVO automated platform therefore offers a solution to laboratories looking to increase throughput for their important and highly challenging casework samples.

Summary

With the Investigator STAR Lyse&Prep Kit, users can:

- Achieve high yields of PCR-ready DNA from casework samples
- Guarantee the quality of important casework samples with an ISO 18385 forensic-grade kit
- Maximize throughput and use of existing resources with the Tecan Freedom EVO automated platform
- Easily implement the system thanks to established and validated protocols

Ordering Information

Product	Contents	Cat. no.
Investigator STAR Lyse&Prep Kit (400)	For 400 preps of 300 µl each from casework and reference samples: Buffer ATL, Buffer QSL3, Buffer QSW1, Buffer QSW2, Bead Suspension G, Buffer ATE, Proteinase K, Carrier RNA, Q-Card, Handbook	931447
Related Products		
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN Rotor-Gene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
Rotor-Gene Q 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9001660

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.