Developmental validation using the Investigator® STAR Lyse&Prep Kit and KingFisher™ Flex Purification System

Ross McEwan[1], John Haley[1], Vanessa Duchamp[2], Anke Prochnow[2], Mario Scherer[2], Keith Elliott[1]

[1] QIAGEN Limited, Citylabs 2.0, 200 Hathersage Road, M13 0BH, UK
[2] QIAGEN GmbH, 1 QIAGEN Strasse, 40724 Hilden, Germany

Introduction

Modern criminal justice relies increasingly on forensic science to identify suspects and bring about convictions. DNA profiling is the established method of choice for human identification. Consequently, submissions to forensic DNA profiling laboratories are growing, forcing labs to find solutions for maximizing throughput whilst assuring quality and maintaining success rates.

Here we demonstrate that high-throughput sample purification can be achieved on the Thermo Fisher® KingFisher Flex Purification System using the QIAGEN Investigator STAR Lyse&Prep Kit. The purification protocol produces a PCR-ready, inhibitor-free DNA with the potential to increase the capacity of forensic casework laboratories tasked with managing ever increasing workloads.

The Investigator STAR Lyse&Prep Kit

The Investigator STAR Lyse&Prep Kit (Figure 1) is designed for automated purification of total DNA from samples encountered in forensic and human-identity applications. The extraction chemistry uses proven QIAGEN magnetic-particle technology (Figure 2) and provides high-quality DNA suitable for direct use in downstream applications using open liquid handler platforms, such as quantitative PCR amplification or STR analyses, or for storage for later use. Purified DNA is free of proteins, nucleases and inhibitors.

Figure 1. The Investigator STAR Lyse&Prep Kit.

The KingFisher Flex Purification System

The KingFisher Flex Purification System (Thermo Fisher Scientific; cat. no. 5400610) can perform all steps of the sample extraction procedure after lysis according to the pretreatment protocols. Up to 96 samples can be processed with the 96-magnet head on a single batch, enabling flexible, high-throughput sample processing. An open and flexible system lets the customer use any magnetic particle-based kit to meet their application demands. Extraction protocols for 300 µL and 500 µL sample lysate volumes are available, and DNA can be eluted in 30–100 µL low TE buffer.

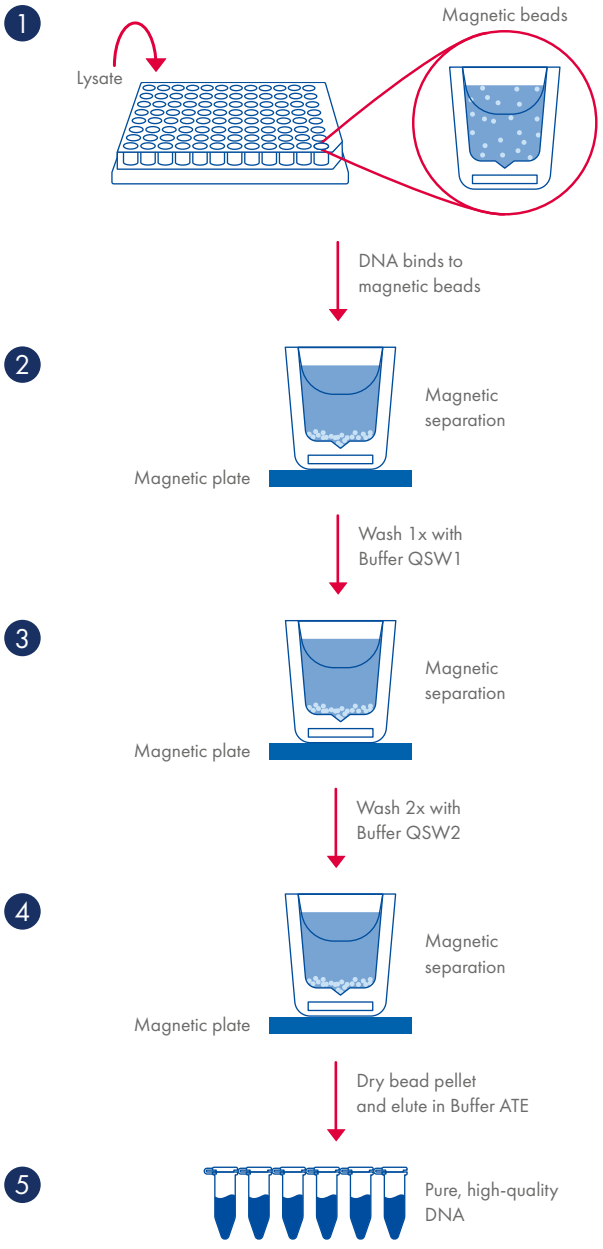

Figure 2. Magnetic bead workflow for the Investigator STAR Lyse&Prep Kit.

Methods and results

Linearity and sensitivity

A 1/5 dilution series was used to determine the range of sample input amounts that can be reliably processed by the KingFisher Flex Purification System using the Investigator STAR Lyse&Prep Kit. The blood series consisted of 5 concentrations of an individual's blood. The saliva series consisted of 5 concentrations of an individual's saliva. Samples of blood and saliva were extracted and quantified in replicates of 8 to assess the quality of the results for each sample type (1). The study was conducted using the Investigator STAR Lyse&Prep Kit 500 µL protocol, and DNA was eluted in 60 µL (2). Samples were quantified using the Investigator Quantiplex® Pro Kit on an Applied Biosystems® 7500 Fast Real-Time PCR cycler (3). The quantification results were analyzed using the QIAGEN Quantification Assay Data Handling and STR Setup Tool v4.1.0.

DNA yields increased in proportion to the amount of sample extracted for blood (Figure 3) and saliva (Figure 4). DNA was efficiently recovered from the lowest concentration replicate tested.

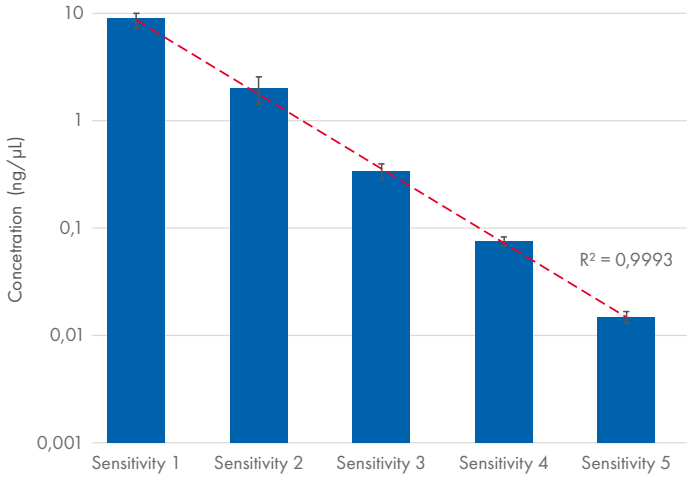

Figure 3. Sensitivity and linearity study for blood. Results obtained for a 1/5 dilution series were comparable between protocols run.

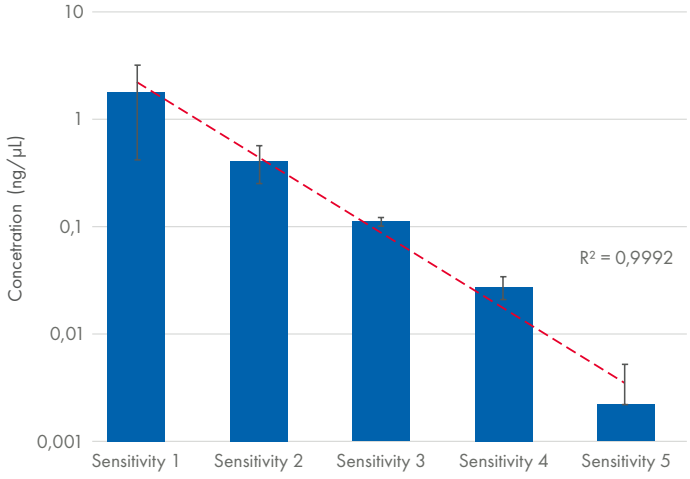

Figure 4. Sensitivity and linearity study for saliva. Results obtained for a 1/5 dilution series were comparable between protocols run.

Stability

Forensic casework samples are frequently associated with potential inhibitors of PCR. These inhibitors must be efficiently removed during extraction to prevent any negative impact on the analysis. The Investigator STAR Lyse&Prep Kit in combination with the KingFisher Flex Purification System was tested for removal of known inhibitors added
to samples.

Soil, dust, blue dye, humic acid (300 ng/µL) and humic acid (800 ng/µL) were spiked into samples containing undiluted saliva. The study was conducted using the 300 µL and 500 µL Investigator STAR Lyse&Prep Kit protocols, and DNA was eluted in 60 µL (2). Samples were quantified using the Investigator Quantiplex Pro Kit on an Applied Biosystems 7500 Fast Real-Time PCR cycler (3). Template of 500 pg DNA per reaction was used for STR PCR with the Investigator 24plex QS Kit (4).

For the inhibited samples processed using the 500 µL protocol, cycle threshold (Ct) values for amplification of the Investigator Quantiplex Pro Kit internal control ranged between 23.76 and 24.94 (Figure 5). For the inhibited samples processed using the 300 µL protocol, cycle threshold (Ct) values for amplification of the Investigator Quantiplex Pro Kit internal control ranged between 24.71 and 26.52 (Figure 6). These results indicate that no inhibition was detected in these samples.

Furthermore, all samples provided full STR profiles without any indication of inhibition (data not shown). These findings of removal of inhibitors and stability were supported by balanced amplification of the Investigator 24plex Quality Sensor QS1 and QS2 (see Figure 7, as an example).

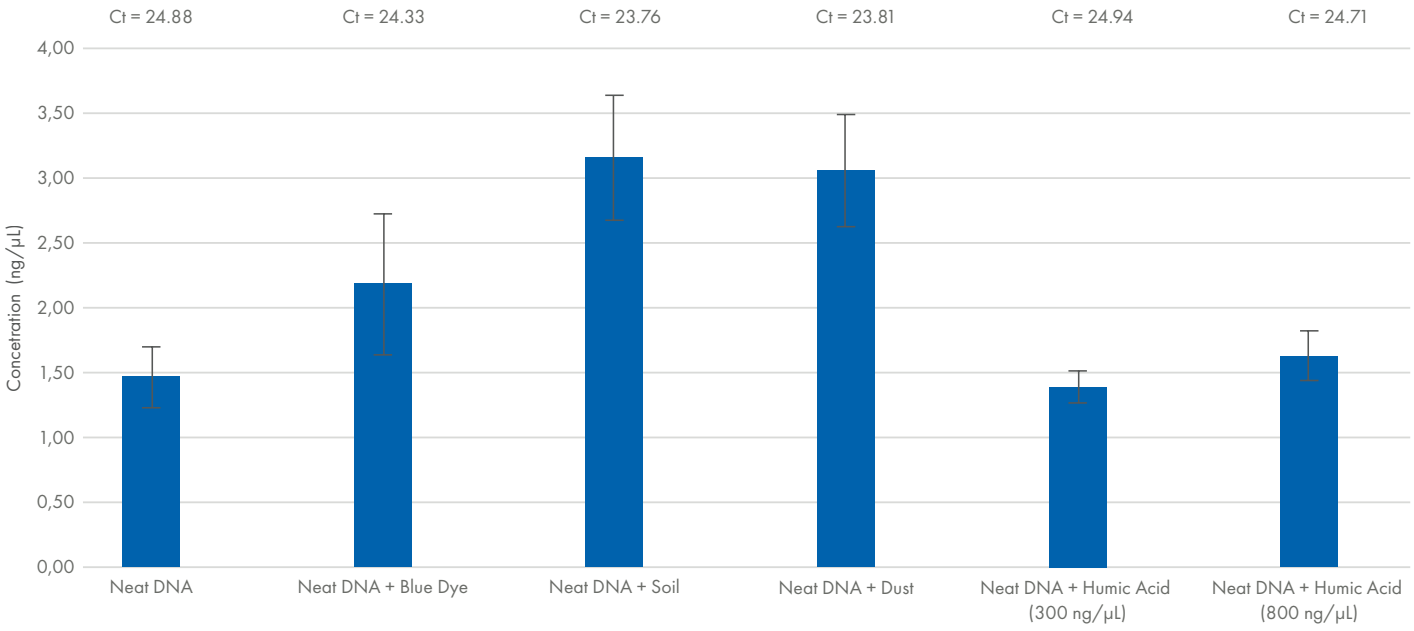

Figure 5. Performance of inhibited samples using the 500 µL protocol. Blue dye, soil, dust, humic acid (300 ng/µL) and humic acid (800 ng/µL) were spiked into neat saliva. Samples were processed in 6 replicates. Sample DNA concentration and the Ct values for the Quantiplex Pro Kit internal control are shown.

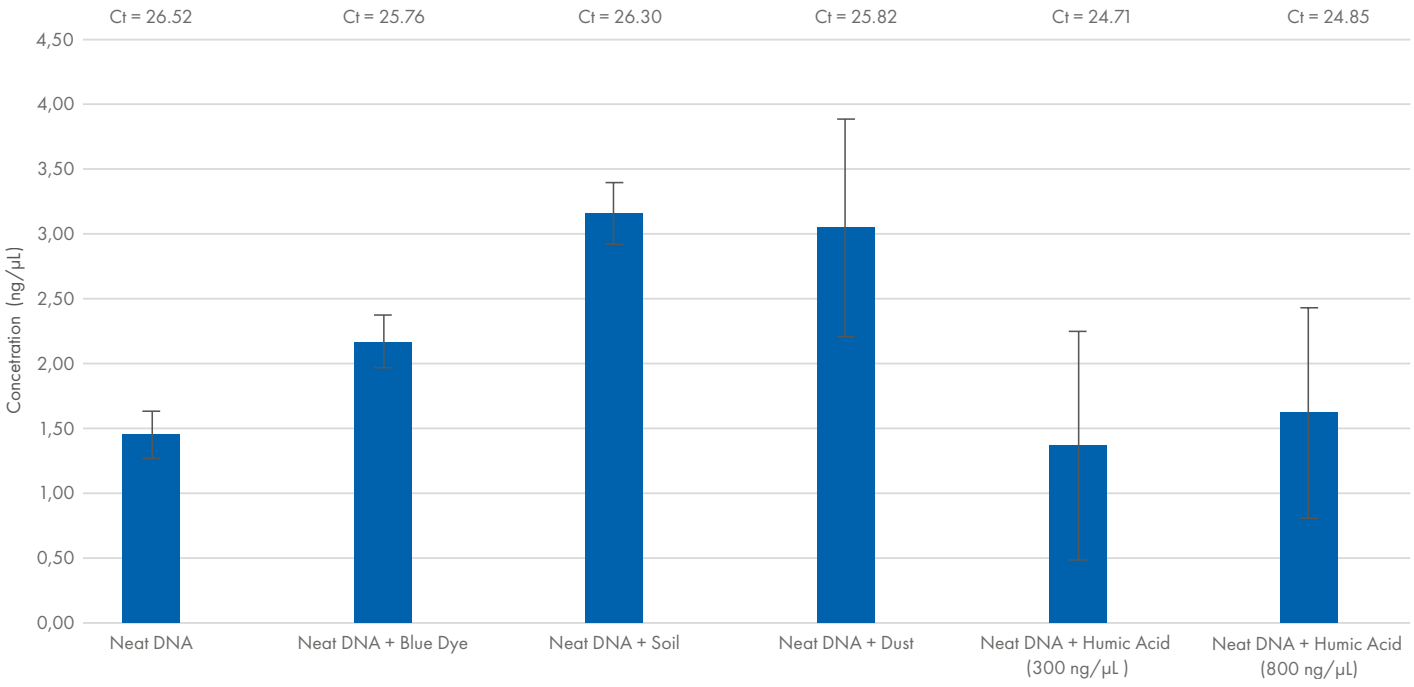

Figure 6. Performance of inhibited samples using the 300 µL protocol. Blue dye, soil, dust, humic acid (300 ng/µL) and humic acid (800 ng/µL) were spiked into neat saliva. Samples were processed in 6 replicates. Sample DNA concentration and the Ct values for the Quantiplex Pro Kit internal control are shown.

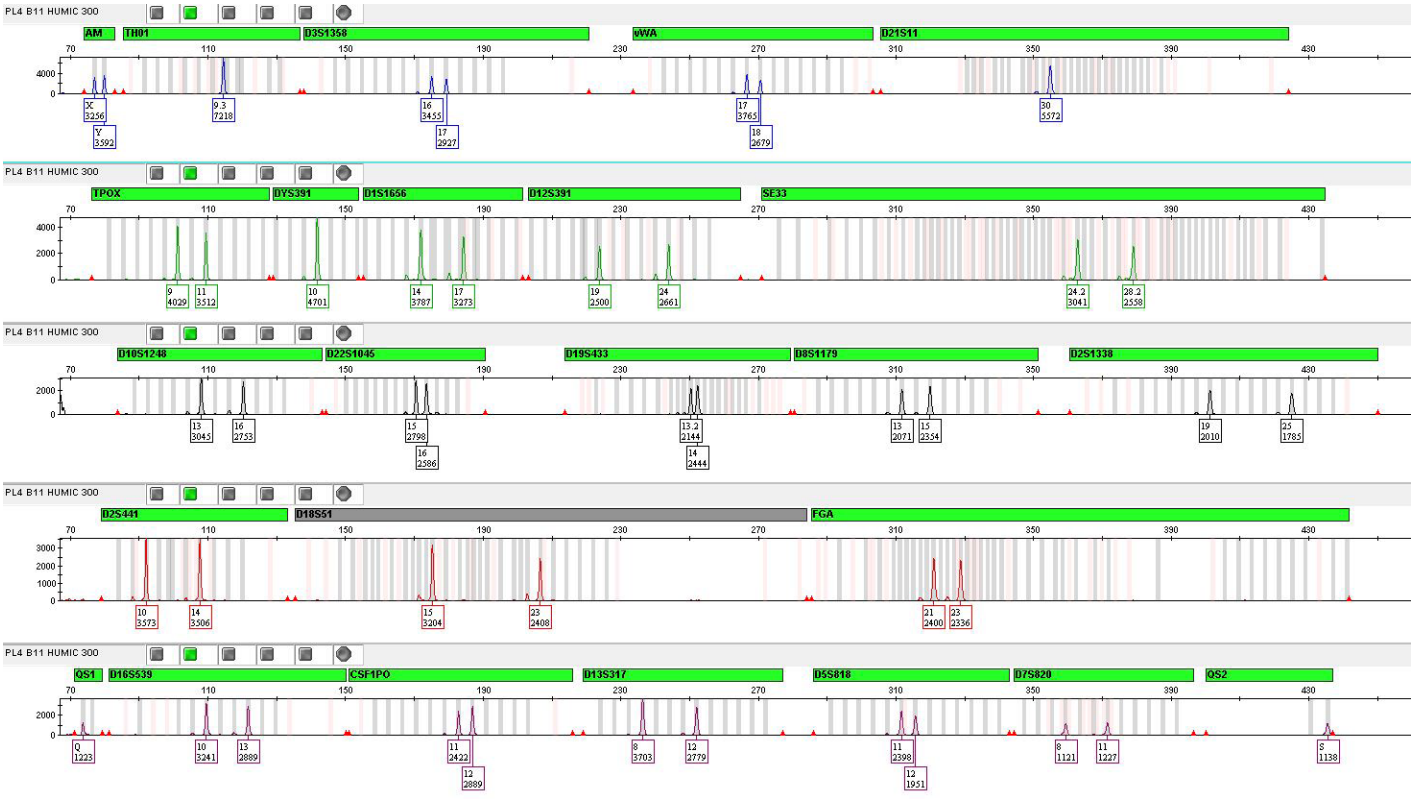

Figure 7. Example EPG with inhibitor. DNA profile for sample spiked with humic acid (300 ng/µL) showing balanced amplification of the Investigator 24plex QS. The Quality Sensors QS1 and QS2 show no indication of inhibition following extraction using Investigator STAR Lyse&Prep on the KingFisher Flex Purification System.

Repeatability and reproducibility

To test repeatability and reproducibility of the extraction, recovery of DNA was determined in 3 runs performed over different days. Eight samples were used in each run for a total of 24 samples. The study was conducted using the KingFisher Flex Purification System and the Investigator STAR Lyse&Prep Kit 500 µL protocol. DNA was eluted in 60 µL (2). Samples were quantified using the Investigator Quantiplex Pro Kit on an Applied Biosystems 7500 Fast Real-Time PCR cycler (3).

Results were comparable between and within groups, demonstrating reliability of the Investigator STAR Lyse&Prep chemistry in combination with the KingFisher Flex Purification System to consistently provide high-quality eluates of DNA (Figure 8).

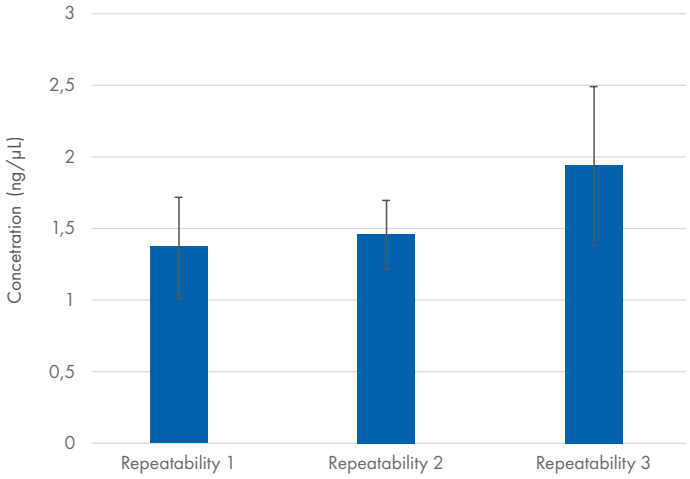

Figure 8. Comparable repeatability and reproducibility. Results were comparable between and within groups, demonstrating reliability of the Investigator STAR Lyse&Prep chemistry to consistently provide high-quality eluates of DNA. Samples were processed in 8 replicates.

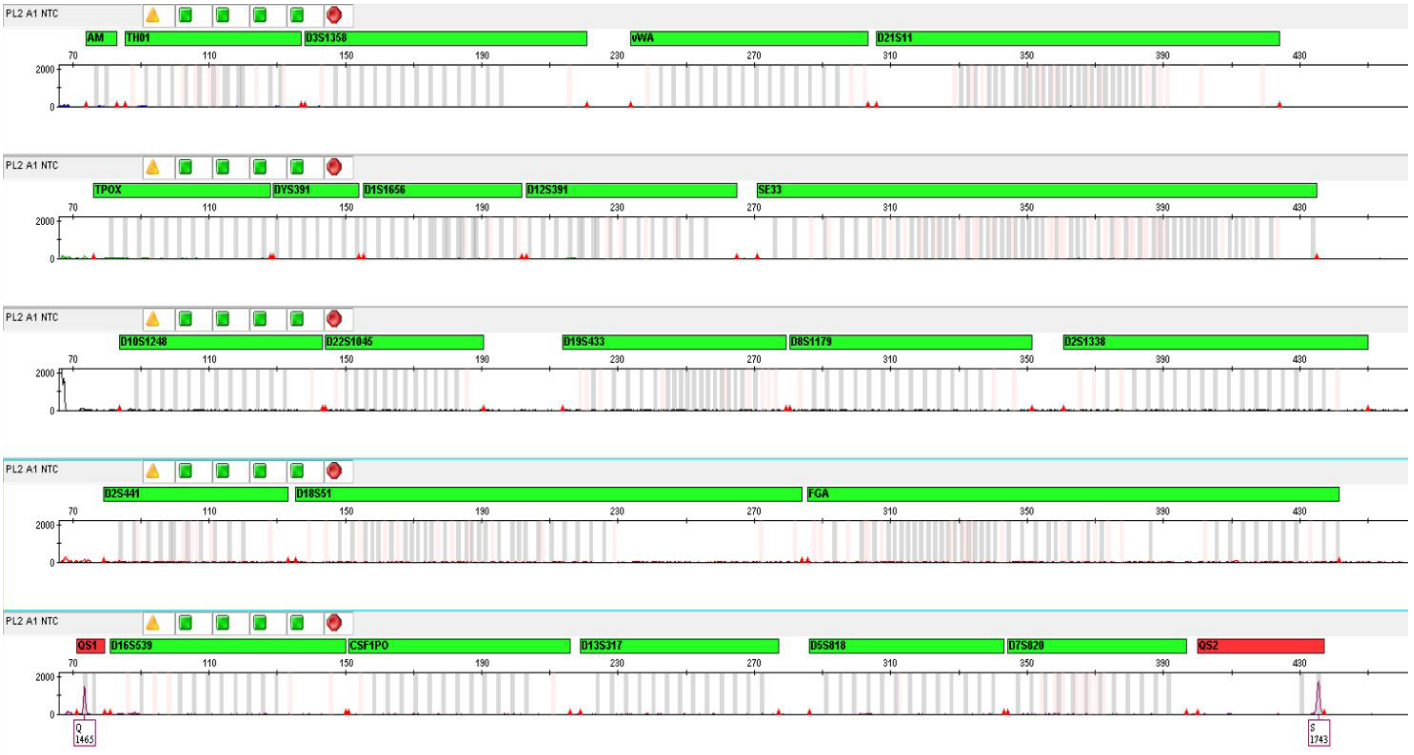

Figure 9. Example electropherogram for a negative sample (NTC). Note that there was no amplification of any allelic peaks throughout all colors. Balanced amplification of both Quality Sensor fragments was shown indicating successful amplification.

Cross-contamination

A contamination study was performed with the Investigator STAR Lyse&Prep 300 µL and 500 µL protocols in combination with the KingFisher Flex Purification System using saliva samples arranged in a checkerboard pattern on a 96-well plate with alternating negative extraction controls. All negative samples were quantified using the Investigator Quantiplex Pro Kit (3). Any negative samples with a quantification value, i.e., not undetermined, were amplified using the Investigator 24plex QS Kit with 15 µL as template (4). No spurious peak was verified (see Figure 9, as an example).

Mock casework samples

A selection of mocked casework samples was processed. Samples were extracted using the Investigator STAR Lyse&Prep 500 µL protocol with 50 µL elution (2) using both the KingFisher Flex Purification System (1) and, for comparison, the QIAGEN QIAsymphony® SP instrument (5). Samples were quantified using the Investigator Quantiplex Pro Kit on an Applied Biosystems 7500 Fast Real-Time PCR cycler (3).

Samples were assigned as "paired" samples as best as possible, with half of each mocked sample processed on each instrument. Comparable DNA yield was achieved for all samples with both instrument systems. The samples were saliva on swabs (Figure 10), blood on swabs (Figure 11), cigarette butts (Figure 12), blood on swabs with potential inhibitors (soil and oil), blood on fabric (Figure 13) and surface swabs from computer mouse and phone (Figure 14).

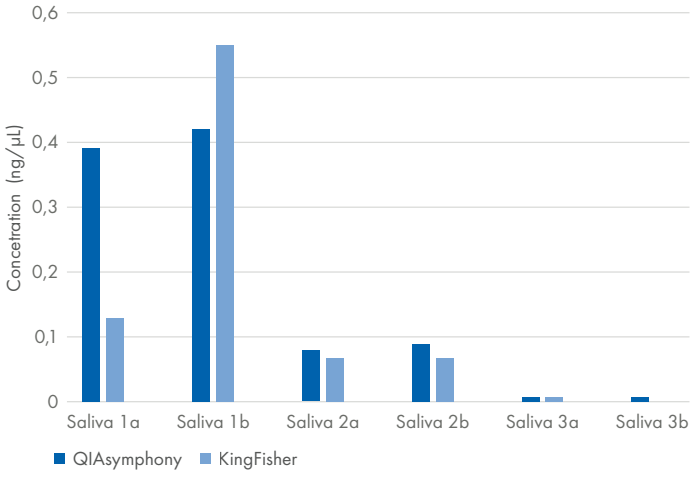

Figure 10. Mocked casework samples: Swabs containing saliva. Swabs were extracted with the Investigator STAR Lyse&Prep Kit on the QIAsymphony SP and KingFisher Flex Purification System.

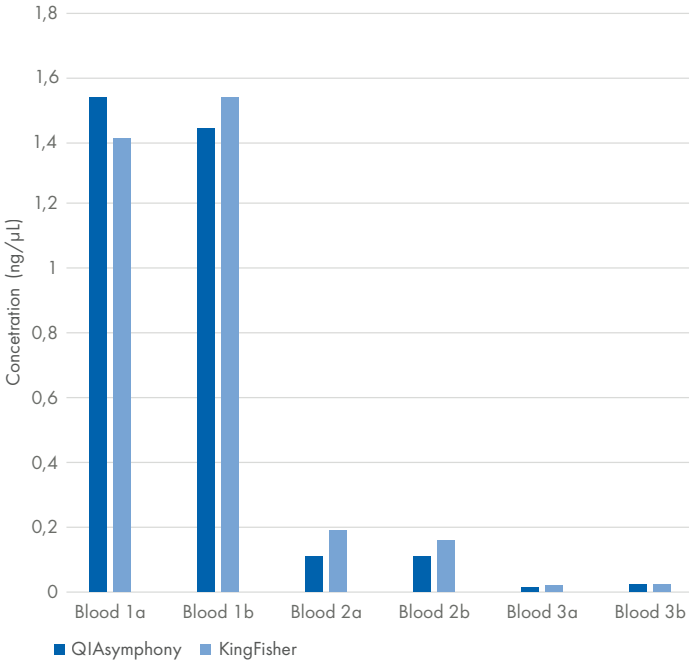

Figure 11. Mocked casework samples: Swabs containing blood. Swabs were extracted with the Investigator STAR Lyse&Prep Kit on the QIAsymphony SP and KingFisher Flex Purification System.

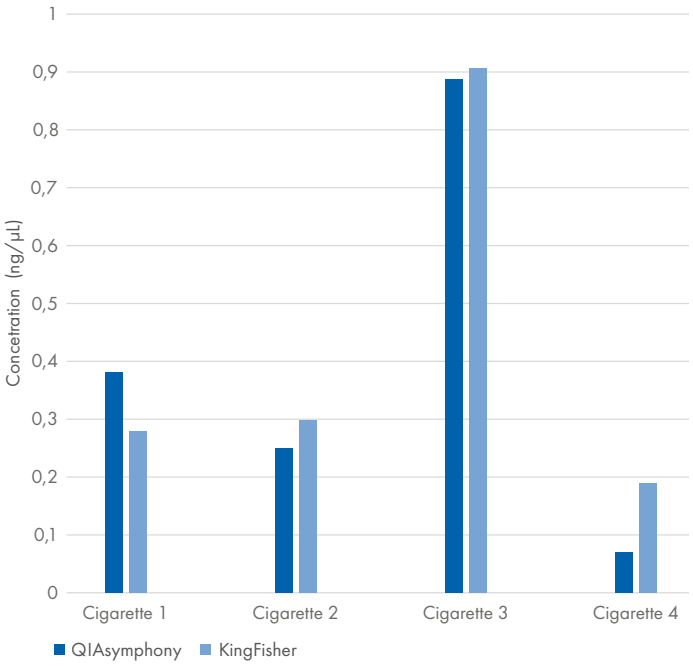

Figure 12. Mocked casework samples: Cigarettes. Samples were extracted with the Investigator STAR Lyse&Prep Kit on the QIAsymphony SP and KingFisher Flex Purification System.

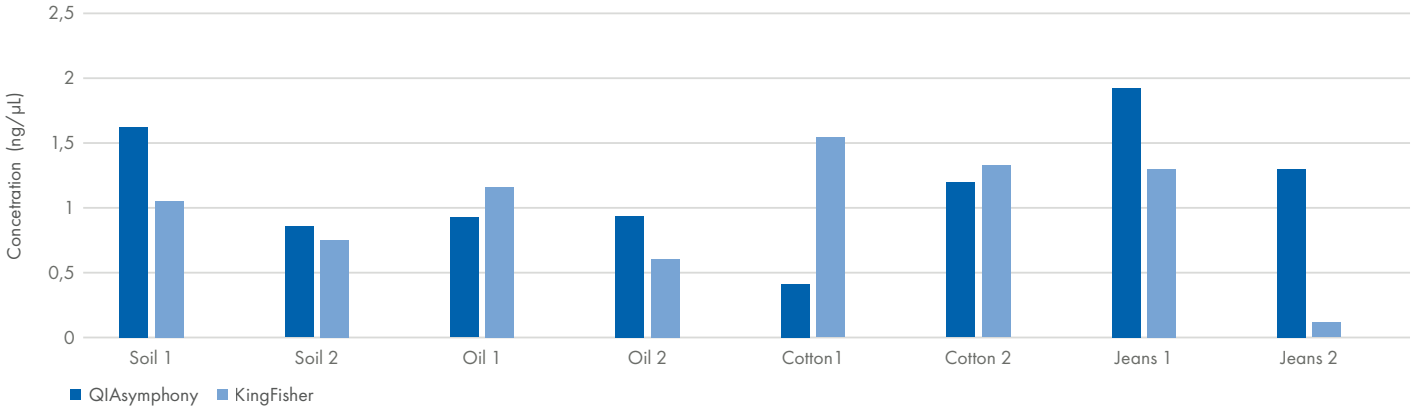

Figure 13. Mocked casework samples: Swabs containing blood mixed with inhibitors and blood on fabric. Swabs were extracted and processed with the Investigator STAR Lyse&Prep Kit on the QIAsymphony SP and KingFisher Flex Purification System.

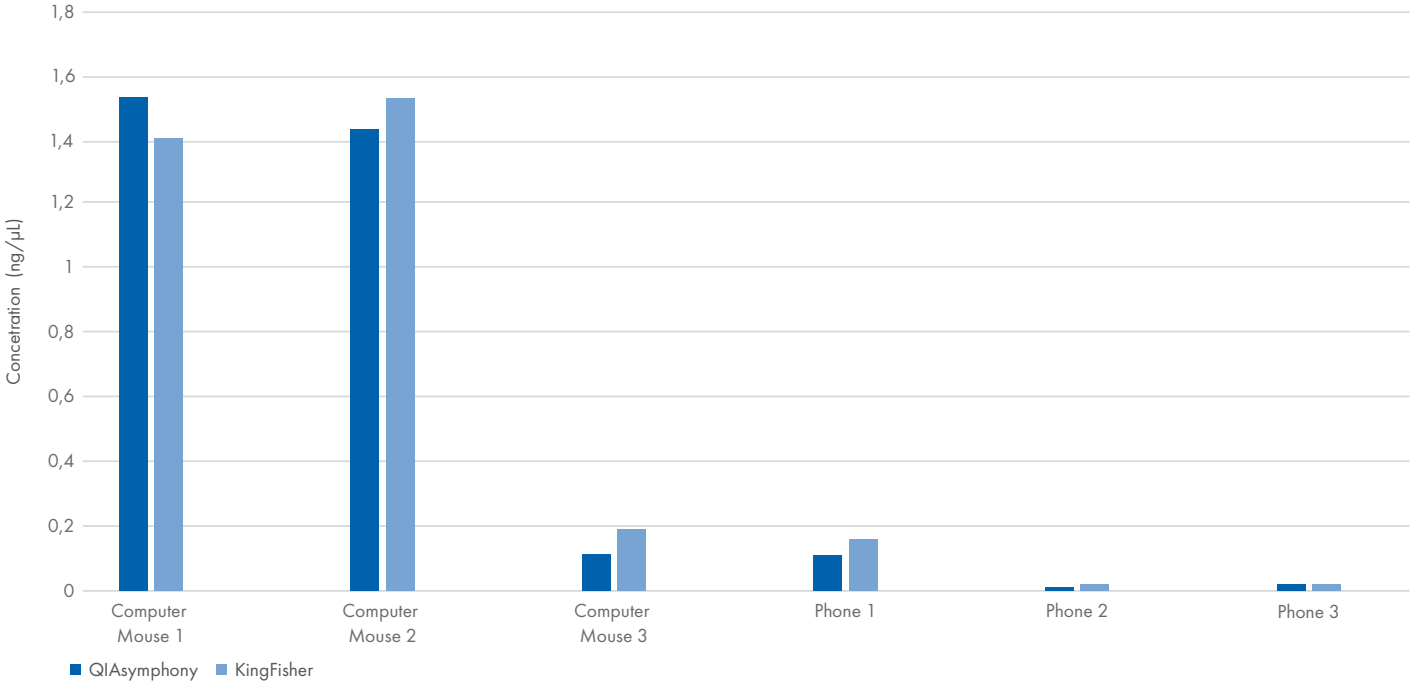

Figure 14. Mocked casework samples: Surface swabs. Swabs were extracted and processed with the Investigator STAR Lyse&Prep Kit on the QIAsymphony SP and KingFisher Flex Purification System.

Conclusion

The Investigator STAR Lyse&Prep Kit meets ISO 18385 requirements and is a 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 KingFisher Flex Purification System, enabling high high-throughput processing of casework samples with no compromise on quality or success rates. The workflow presented here using the Investigator STAR Lyse&Prep Kit in combination with the KingFisher Flex Purification system therefore offers a solution to laboratories looking to increase throughput for their important and highly challenging casework samples.

Summary

- The Investigator STAR Lyse&Prep Kit meets ISO 18385 requirements to guarantee the quality of your important casework samples.
- You can maximize throughput and use of your existing resources with the KingFisher Flex Purification System.
- Established and validated protocols make implementation easy in your laboratory.

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
Investigator Quantiplex Pro Kit (200)	For use on Applied Biosystems Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387216
Investigator 24plex QS Kit (100)	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO), and nuclease-free water	382415
Investigator 24plex QS Kit (400)	Primer mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO), and nuclease-free water	382417

Learn more about the Investigator STAR Lyse&Prep Kit for your lab. Visit **qiagen.com/STARLyse&Prep**

References

1. *KingFisher™ Flex Instructions for Use (Protocol Sheet).* September 2022.
2. *Investigator STAR Lyse&Prep Kit Handbook.* August 2022
3. *Investigator Quantiplex Pro Handbook for Applied Biosystems 7500 Real-Time PCR Systems.* January 2023.
4. *Investigator 24plex QS Handbook.* February 2021.
5. *QIAsymphony SP/AS – Operating the QIAsymphony SP.* December 2017.