Application Note

High-Throughput DNA Extraction from Forensic Trace Samples Using QIAGEN Investigator® Chemistry and the Hamilton® AutoLys

Bas de Jong[1], Sander Kneppers[1], Keith Elliott[2], Natalie Tyler[2] Anke Prochnow[2]

[1] Netherlands Forensic Institute, The Hague, The Netherlands

[2] QIAGEN GmbH, Hilden, Germany

We describe the development and validation of a protocol for high-throughput sample preparation of forensic casework samples, including automated sample lysis, using QIAGEN Investigator magnetic bead chemistry and Hamilton's Microlab® Autolys STAR system. This protocol provides high success rates for 95% of all sample types, enabling automation of almost all sample submissions with a single protocol. This represents a new model of efficiency in high-throughput forensic laboratories.

Introduction

Modern forensic ISO/IEC-17025 accredited laboratories are faced with the challenge of increasing demand to expand throughput, while maintaining the highest possible success rates and quality at an acceptable cost level. Improved throughput can be achieved with the use of automation platforms. However, this comes at the cost of reduced flexibility, for example, when changing protocols for different sample types. The department of Human Biological Traces of the Netherlands Forensic Institute (NFI) receives 40,000 casework samples for DNA profiling each year, including 17,000 volume crime samples (e.g., automobile thefts) and 23,000 serious crime samples. The type of samples submitted to the laboratory varies significantly, with touch DNA, body fluids and many other samples, all received on a wide range of substrates. These substrates also vary considerably and can include multiple inhibitors and other substances problematic to DNA profiling. Together these factors present a unique challenge to high-throughput forensic laboratories, if high success rates are to be maintained: the various demands presented by each sample are counteracted by the need for simple streamlined workflows. In addressing this challenge, the NFI has developed a single high-throughput workflow based on QIAGEN Investigator chemistry and the Hamilton Microlab Autolys STAR system (hereafter "AutoLys system"). This workflow enables robust, fully automated, on-instrument lysis and purification, ensuring high-quality recovery of amplifiable DNA from almost all sample types.

Materials and methods

In this extensive validation study, the NFI tested a variety of mock casework samples. These samples ranged from dilutions of body fluids spotted on a variety of substrates, to used or consumed items such as cigarettes and chewing gum. For an overview of tested samples, see Table 1. Samples were either processed on the AutoLys system using QIAGEN Investigator magnetic bead chemistry or extracted manually using the QIAGEN QIAamp® DNA Mini Kit and QIAshredder columns.

Manual and fully automated lysis followed a similar protocol for this study: all samples were pre-heated at 85°C for 10 minutes before adding proteinase K. According to an in-house protocol, samples were then incubated for 4 hours at 56°C. For the manual extraction, this was followed by the steps described in QIAGEN's regular protocol. The automated extraction followed an in-house optimized protocol, including three washing steps and elution in 100 µl.

All DNA extracts were quantified using an in-house developed method based on Alu-repeats and subsequently amplified using a commercially available 17 STR marker kit, followed by fragment separation on an Applied Biosystems® 3130xl Genetic Analyzer.

Results

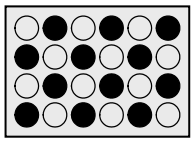
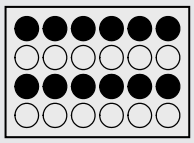
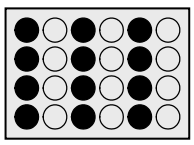
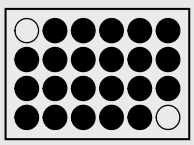
The manual extraction method was chosen as a benchmark due to its high success rate, which the NFI wanted to replicate in an automated solution. Therefore, results are presented as yields from the AutoLys system versus yields from manual extraction. These were calculated from the average peak heights from the DNA profiles and corrected for the amount of DNA extract used as template DNA in the PCR reaction. By averaging over the series for a particular sample type and comparing to the same calculation over a series extracted manually, we determined a percentage representing the yield: 100% means similar yield between methods, while a result of less than or greater than 100% corresponds to a lower or higher yield, respectively. Results are summarized in Table 1.

Table 1. Comparison of yields from the AutoLys system versus yields from manual extraction

Sample type* (n=5)	Swab	Polyester	Denim
Blood (high)	93% (1.244 ng/µl)	54% (0.976 ng/µl)	92% (0.622 ng/µl)
Blood (low)	95% (0.048 ng/µl)	90% (0.026 ng/µl)	103% (0.017 ng/µl)
Saliva (high)	67% (0.898 ng/µl)	78% (0.205 ng/µl)	71% (0.443 ng/µl)
Saliva (low)	116% (0.023 ng/µl)	57% (0.013 ng/µl)	115% (0.011 ng/µl)
	Cigarette butt	**Chewing gum**	**Tape-lifted contact traces**
Normal usage	89% (0.463 ng/µl)	208% (0.122ng/µl)	86% (0.113ng/µl)

A test for cross-contamination was performed, as well, in which samples were prepared in a specific order to detect cross-contamination: checkerboard, horizontal zebra and vertical zebra patterns. Additionally, two blanks were co-extracted with each test run during the validation study. Setups and results are summarized in Table 2.

Table 2. Experimental setup and results of cross-contamination analyses

Cross-contamination check	Plate layout	Results complete procedure
A: Checkerboard		0% (0/200+ samples)
B: Horizontal		0% (0/500+ samples)
C: Vertical		0% (0/500+ samples)
D: Blanks		0% (0/500+ samples)

An additional analysis was performed on the quality of the DNA profiles: background noise, intra- and interlocus peak balance and the abundance of artifacts were analyzed. For all of these quality markers, no differences between automated extraction on the Hamilton AutoLys system and manual extraction were found.

Benefits

Using the high-quality QIAGEN chemistry on the AutoLys system enables the NFI to process over 95% of their forensic samples in an automated manner. This means that over 35,000 samples which would previously have been processed manually or semi-automated at sub-optimal efficiency, are now processed at the high-quality level of manual DNA extraction. At the same time, the fully automated protocol drastically reduces the risk of cross-contamination and sample misplacements, thus increasing the probative value of the forensic samples.

At the NFI, the AutoLys system has increased the number of samples that could be processed at a time, while decreasing operator time per sample. Previously, two employees were needed for every batch of 36 samples – now, one analyst operates three systems, each processing 96 samples.

NFI continuously reviews their protocols and since implementation has managed to reduce the overall lysis time to two hours while still achieving the same goals as initially outlined.

Conclusions

- QIAGEN Investigator chemistry can be successfully used with the AutoLys system to enable automation of lysis and purification of almost all forensic samples with a single protocol.
- Success rates for this protocol are comparable with the previously validated manual method (data not shown) and better than the semi-automated methods for processing these samples at the NFI.
- This workflow represents a new model of efficiency for high-throughput forensic laboratories, enabling a single, highly streamlined process for almost all samples, without compromising success rates.

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.

Find out more at **www.qiagen.com/InvestigatorIDKits**.