Abstract 80

AUTOMATED HIGH-THROUGHPUT FORENSIC DNA EXTRACTION USING THE DNA IQ™ SYSTEM AND SLICPREP™ 96 DEVICE FROM PROMEGA AND THE JANUS® FORENSIC WORKSTATION FROM PERKINELMER

Diane Komonski[1], Scott Myhal[1], Al Marignani, Roger Frappier[1], John Balog[2], Gary Reznik[3] and Lois Tack[3]
[1]Centre of Forensic Sciences, Toronto, ON
[2]PerkinElmer Life and Analytical Sciences, Woodbridge ON
[3]PerkinElmer Life and Analytical Sciences, Downers Grove IL

The Centre of Forensic Sciences (CFS) in Toronto has processed "break and enter" (B&E) and other high volume type casework samples for several years. To accommodate the large sample workload, two robotic liquid handling systems from PerkinElmer were used to automate a set of four DNA processing protocols in 96-well format. These included (1) DNA isolation using the Promega DNA IQ™ System, (2) DNA quantification using real-time quantitative PCR (QPCR) analysis, (3) DNA normalization and dilution, and (4) STR-typing amplification setup. Recently, a third PerkinElmer liquid handling system, the JANUS® Forensic Workstation, was added. The intent was to support current implemented automated protocols but also to begin validating new procedures being evaluated for automated casework sample processing. The first method chosen for the new JANUS system was low-abundant DNA extraction from "touch" swabs and other difficult-to-extract sample types, including "drink container swabs" (DCS). Fully automated on-deck DNA extraction of touch samples using a PerkinElmer robot and the Slicprep™ 96 Device from Promega had been reported by Earl Ritzline [1].

We describe here initial validation of a similar, further enhanced automated DNA extraction protocol using the Slicprep Device and a JANUS two-arm platform with a fully integrated PerkinElmer dual-temperature heating and IKA shaker system. The workstation carried out on-deck sample extraction in the Slicprep spin basket-extraction plate device using DCS, other touch swabs, cigarette butts, buccal swabs and blood-stained punches. The device was placed on preheated heater tile(s) located on an IKA KS130 4-plate capacity shaker placed directly on the JANUS grid deck. WINPREP® software directed the addition of 400 uL lysis buffer and a 30 min concomitant heating and shaking extraction procedure. A custom heat block insert, designed to precisely fit the Slicprep 96 deep well plate, provided even heating of samples with a 90 C set temperature. The extraction device was removed from the deck, a spacer collar inserted to raise the spin basket, and centrifuged for 5 min at 1500 x g. After centrifugation, the spin basket insert and collar were removed and the extraction plate placed on the robot deck for processing. Before centrifugation, with spin baskets raised above the extraction fluid, nearly half the extraction fluid was absorbed by full-sized cotton swabs. After centrifugation, quantitative "stripping" of extraction buffer from swabs was observed. The JANUS robotic system then carried out automated DNA purification (binding, washing and elution steps) using DNA IQ™ System reagents and the same shaker and heater system. The DNA elution temperature was set at 85 C. Sample quantity and quality was assessed using QPCR and STR amplification setup methods.

Checkerboard pattern analysis indicated the samples to be cross-contamination-free. Yields were consistent with manual and other automated purification methods [2]. Automating the front-end extraction steps with the Slicprep device, thereby removing most sample pre-processing, virtually eliminates any manual liquid handling steps. The only manual user step used was centrifugation. This approach also eliminates substrate carryover noted for some casework sample types. Introduction of an automated on-deck Slicprep extraction protocol results in more robust casework sample DNA processing, decreased user sample handling and improved casework sample throughput.

References

[1] E. Ritzline, Indian River Crime Lab, Fort Pierce FL. May, 2007, Personal Communication,

[2] A. Tereba, J. Krueger, R. Olson, P. Mandrekar and B. McLaren. "Profiles in DNA", September 2005, pp 3-5.