

A FULLY AUTOMATED DNA IQ™ PURIFICATION METHOD FOR LIKELY HIGH DNA YIELD CASEWORK SAMPLES ON A TECAN® FREEDOM EVO® WORKSTATION.

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In our laboratory casework samples of all types are extracted and purified manually using a modified DNA IQ™ (Promega) protocol. We have now adapted our protocol on a Tecan® Freedom EVO® workstation equipped with a fixed tip liquid handler and a disposable tip MultiChannel Arm™. We have validated a fully automated method for processing of casework samples with likely high DNA yield, including blood, saliva, sperm and epithelial cell fractions.

The Freedom EVO® workstation is equipped with a liquid handler (LiHa) arm bearing eight fixed tips, a MultiChannel Arm™ (MCA) with a 96-disposable tip head, a robotic manipulator (RoMa) arm, an integrated plate sealer (Abgene), a microplate centrifuge (Velocity11), a 2D code reader (Bio Meridian), a Te-shake™ plate shaker, as well as two magnet stations and a heating block. This workstation is integrated with our in-house LIMS (DNA Pro-FILES, Synchrone InfoSystème), which generates worklists used by the Freedom EVOware® software for automated preparation of the purification plates and final transfer of purified DNA in Matrix microtubes for storage.

DNA content of forensic casework samples varies widely, from a few ng to tens of µg of total DNA. We have validated our automated protocol for processing lysates containing up to 75 ng/µl of DNA. The risks of contamination are minimized by working with low volume, performing key steps in closed/sealed containers, and optimizing arm movements over the sample plates. The proteinase K lysis and filtration steps are performed manually, after which the lysate is stored in a Thermo Scientific MATRIX® 2D barcoded minitube equipped with a Septra Seal® cap until processing on the workstation. The lysate is then transferred by the LiHa to its corresponding position in a 96-deepwell plate already containing the Promega lysis buffer and magnetic beads. The plate is sealed with an easy-pierce foil seal followed by incubation at room temperature on the shaker. The plate is then transferred to a pin magnet and the supernatant is discarded using the 96-tips MCA. All subsequent purification steps (washing, elution) are also carried out using the MCA. After elution, DNA is transferred by the MCA in a 96-well microplate positioned on a ring magnet. Finally, the eluted DNA is transferred by the LiHa into Thermo Scientific MATRIX® 2D barcoded minitubes equipped with Dura Seal® plugs. A single plate is processed in approximately 2 hours.

The operational scripts are optimized to maximize throughput without compromising DNA yield and quality, as well as processing reliability. Our validation parameters included optimization of DNA yield and DNA quality as well as investigation of sample to sample contamination risks. Blanks (96) extracted with highly concentrated blood samples (up to 75 ng/µl) in checkerboard configuration showed no alleles upon amplification of 10% of the blank volume. When the total blank volume was concentrated and amplified, only rare alleles barely above detection levels (80 rfu) were observed in a few wells. This is comparable to the background of the manual method. DNA yield and quality from blood samples (90), mock forensic samples (66) and non-probative forensic samples (13) were assessed using a direct sample to sample comparison: for each sample, one portion of the lysate was purified manually and another portion was purified on the workstation. We obtained comparable DNA yield and profile quality with both methods.