MAXWELL® 16 LEV

The Maxwell® 16 Low Elution Volume System for Forensic Casework

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We have developed an affordable, high-quality automated instrument that is optimized for DNA extraction from forensic casework samples and allows the use of low elution volumes.

INTRODUCTION

Isolation of genomic DNA for short tandem repeat (STR) analysis from casework samples presents a number of challenges to the forensic examiner. Sample inputs can include such varied materials as fabric, chewing gum or a forensic contact swab from a crime scene. Samples with little available DNA must be extracted using a method that maximizes the amount of DNA recovered but elutes in a small volume. Promega has developed and optimized the Maxwell® 16 Instrument to process samples in a low elution volume (LEV) automated format to address these concerns. The Maxwell® 16 LEV method for casework samples uses the DNA IQ™ chemistry, which is accepted in the forensic community for reliable isolation of DNA from forensic samples and has been employed to process forensic casework samples in manual and automated formats.

MAXWELL® 16 LEV SYSTEM

A new instrument in the Maxwell® 16 family has been developed for forensic casework samples, where DNA quantities are limited and final concentrations are important. The Maxwell® 16 LEV System is an integrated system that complements the standard elution volume (SEV) Maxwell® 16 System and allows scientists to process up to 16 casework samples and concentrate DNA into 25–50 µl final volumes for downstream analysis. This instrument uses plastic plungers, magnets, elution tubes and reagent cartridges that are designed to work in tandem to process large sample volumes and enable small elution volumes (Figure 1). Reagents are predispensed into disposable cartridges, and the provided 0.5 ml elution tubes are attached to each cartridge. The elution tube can be removed, capped and stored immediately following the automated procedure.

The Maxwell® 16 Instrument is available preconfigured in the SEV or LEV configuration, and it is easy to convert a Maxwell® 16 SEV Instrument to the LEV configuration. Within a few minutes, a new user can convert a Maxwell® 16 SEV Instrument to a Maxwell® 16 LEV Instrument and back again with the use of the Low Elution Volume (LEV) Hardware Kit for Maxwell® 16 (Cat.# AS1250). The Maxwell® 16 Instrument is low maintenance. It remembers its setup configuration and uses an automated diagnostic procedure, which occurs automatically before, during and after each run, to ensure that the instrument is in alignment.

FORENSIC CASEWORK SAMPLE EXTRACTION

Previously, we described DNA extraction from swabs with the Maxwell® 16 System in the context of forensic database applications (1). While the processing of samples is similar, forensic casework samples are likely to have less DNA and thus require greater emphasis on maximizing DNA yield. To quantitate DNA from such DNA-limited samples, we chose the Plexor® HY System, a quantitative real-time PCR method that detects extremely small amounts of DNA.

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Figure 1. The Maxwell® 16 Low Elution Volume plasticware. The plasticware is designed to accommodate large sample input volumes and low elution volumes.

SAMPLE TYPE CONSIDERATIONS

Genomic DNA yields obtained with the DNA IQ™ System are consistent when the available DNA is in excess of the DNA-binding capacity of the DNA IQ™ Resin. Alternatively, for samples with small amounts of DNA, such as many forensic casework samples, the binding capacity of the DNA IQ™ Resin should be significantly greater than the available amount of DNA, providing efficient capture of DNA.

Liquid blood and blood stains contain a higher amount of protein than other samples. Protein can bind to the DNA IQ™ Resin particles and compete with DNA for binding. To reduce the competition, we added a proteinase K treatment to degrade proteins and prevent their interaction with the DNA IQ™ Resin. Additionally, the proteinase K step may increase the removal of cells from a matrix such as a cotton swab. We have demonstrated a significant improvement in DNA yield from forensic samples with a proteinase K preprocessing step (2).

COMPARATIVE DNA EXTRACTION EXPERIMENT

Liquid blood was diluted 1:100 in phosphate buffered saline (PBS), and 100 µl was placed onto each cotton swab. Swabs were allowed to dry overnight at room temperature. DNA was extracted using the DNA IQ™ Casework Sample Kit for Maxwell® 16(i) (Cat.# AS1210), EZ1 DNA investigator kit and iPrep™ Charge Switch® forensic and buccal cell kits as described below.

DNA IQ™ Casework Sample Kit for Maxwell® 16

Each swab was incubated with 90 µl of Incubation Buffer [from the Tissue and Hair Extraction System (for use with DNA IQ™), Cat.# DC6740] and 10 µl of 18 mg/ml proteinase K for 1 hour at 56°C. Three hundred microliters of DNA IQ™ Lysis Buffer was added, and each sample was vortexed briefly. Samples were centrifuged through a DNA IQ™ Spin Basket at 14,000 × *g* for 2 minutes in a microcentrifuge to separate the lysate from the solid support. Lysates were then processed directly with the DNA IQ™ Casework Sample Kit for Maxwell® 16 cartridges. The resulting DNA was eluted into 50 µl of Elution Buffer.

EZ1 DNA Investigator Kit

Each swab was incubated as described in the *EZ1 DNA Investigator Handbook* (Pretreatment for Forensic Surface and Contact Swabs followed by the Trace Protocol without the addition of carrier tRNA) using the EZ1 DNA investigator card installed on a BioRobot® EZ1 workstation. The resulting DNA was eluted in 50 µl of TE buffer as per manufacturer's recommendation.

iPrep™ Charge Switch® Forensic and Buccal Cell Kits

Each swab was treated as per the iPrep™ Charge Switch® forensic and buccal cell kit protocol for preprocessing, then extracted using an Invitrogen iPrep™ instrument installed with an iPrep™ forensic card; DNA was eluted in 75 µl, as per manufacturer's recommendation.

DNA YIELD

DNA extracted using the three extraction methods was quantified using the Plexor® HY System. The resulting DNA concentrations were multiplied by recovered elution volumes to determine DNA yield from the same input sample types (Figure 2). DNA yields using the Maxwell® 16 LEV Instrument were significantly higher than those obtained using the BioRobot® EZ1 workstation and iPrep™ instrument.

FORENSIC TOUCH SAMPLES

To demonstrate a genotype derived from forensic contact swabs, we used moistened swabs to gather duplicate touch sample types from our laboratory. Swabs were dried overnight. Each swab was incubated with 90 µl of Incubation Buffer (from the Tissue and Hair Extraction System) and 10 µl of 18 mg/ml proteinase K for 1 hour at 56°C. Three hundred microliters of DNA IQ™ Lysis Buffer was added, and each sample was vortexed briefly. Samples were centrifuged through a DNA IQ™ Spin Basket at 14,000 × *g* for 2 minutes in a microcentrifuge to separate the lysate from the solid support. Lysates were processed directly with the DNA IQ™ Casework Sample Kit for Maxwell® 16, and DNA was eluted in 50 µl of Elution Buffer. These samples were quantitated and normalized, and we amplified 0.5 ng using the PowerPlex® 16 System. For samples yielding less than 0.5 ng, we amplified 19 µl, the maximum volume of DNA that can be added to a 25 µl PowerPlex® 16 reaction. The results from a swab sample from a laboratory doorknob processed in this fashion are shown in Figure 3.

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CROSS-CONTAMINATION

We assessed sample-to-sample cross-contamination by testing blank samples interspersed with 5 µl samples of liquid blood. All samples were preprocessed with a proteinase K solution. DNA IQ™ Casework Sample Kit cartridges were loaded onto all 16 positions of a Maxwell® 16 LEV Instrument deck. Liquid blood lysate was loaded into odd-numbered positions. Blank samples were loaded into even-numbered positions. All blanks and blood lysates were processed using the Maxwell® 16 LEV forensic method. Fifteen microliters of each even-numbered extract (presumptive blank samples) was amplified in a standard 25 µl PowerPlex® 16 reaction. No peaks were detected above 50 RFU using an ABI PRISM® 3100 genetic analyzer (data not shown).

CONCLUSIONS

We have developed an affordable, high-quality automated instrument that is optimized for forensic casework and allows the use of low elution volumes. The Maxwell® 16 LEV System can extract DNA from up to 16 samples in approximately 30 minutes after preprocessing using the DNA IQ™ chemistry, a proven system that minimizes contaminants that interfere with downstream processes such as DNA quantification or STR amplification.

This system offers forensic examiners the flexibility to preprocess samples as per existing DNA IQ™ System guidelines to maximize DNA yield. We recommend a proteinase K treatment to increase yield from potentially complex sample types bound to a solid material, such as swabs or fabric. As demonstrated with small volumes of liquid blood, the Maxwell® 16 LEV System can consistently extract more DNA than competitive instrumentation and forensic chemistries.

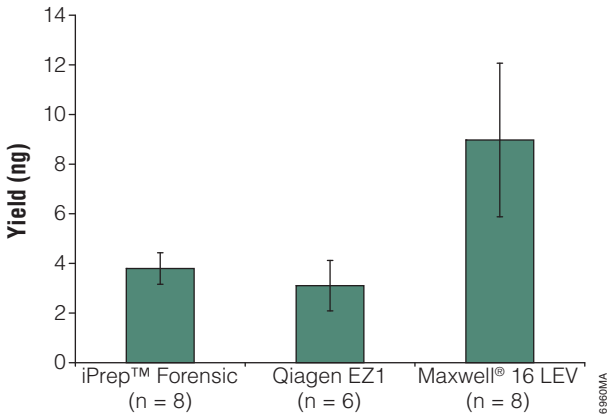

Figure 2. DNA yields from liquid blood. Swabs containing 1 µl of liquid blood were processed using the recommended protocols for the Invitrogen iPrep™ instrument, Qiagen BioRobot® EZ1 workstation and Maxwell® 16 LEV Instrument. Samples were quantified using the Plexor® HY System. Error bars represent one standard deviation.

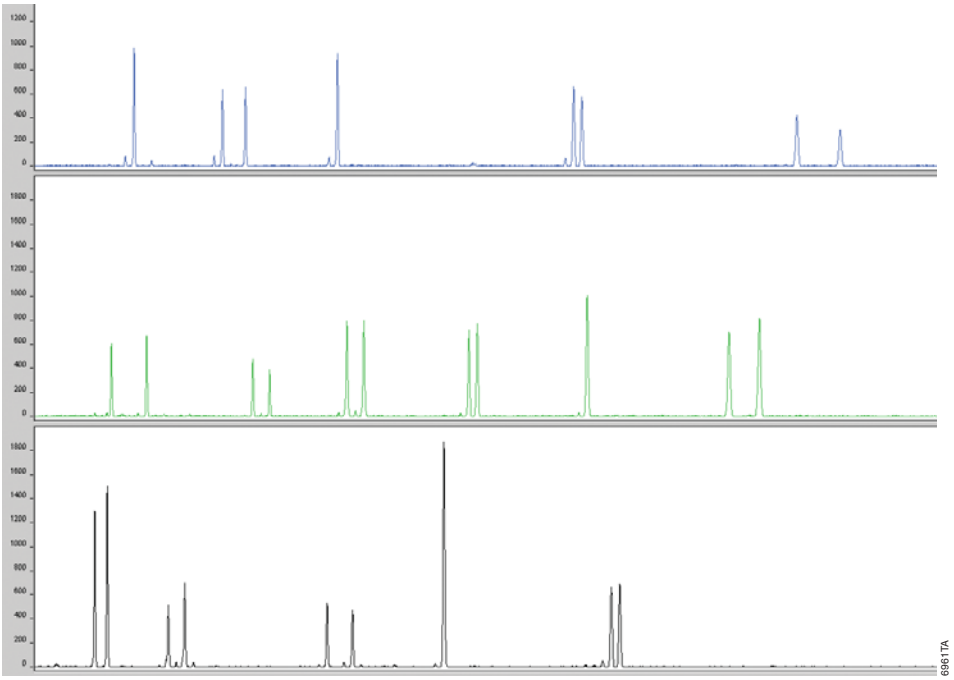

Figure 3. STR analysis of DNA extracted from touch samples. A touch sample was generated from a laboratory doorknob, and DNA was extracted using the DNA IQ™ Casework Sample Kit for Maxwell® 16. The resulting DNA (600 pg) was amplified with PowerPlex® 16 System and analyzed using an ABI PRISM® 3100 genetic analyzer.

REFERENCES

1. Bjerke, M. *et al.* Forensic applications of the Maxwell® 16 Instrument. *Profiles in DNA* **9**(1), 3–5.
2. Mandrekar, P.V., Flanagan, L. and Tereba, A. (2002) Forensic extraction and isolation of DNA from hair, tissue and bone. *Profiles in DNA* **5**(2), 11–13.