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Forensic Applications of the Maxwell™ 16 Instrument

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INTRODUCTION

Isolating genomic DNA for short tandem repeat (STR) analysis is an important, yet time-consuming, process faced by many forensic laboratories each day. The number of samples to be processed continues to climb as new legislation and testing initiatives are implemented. A wide variety of high-quality automated liquid-handling instruments, each with its own unique benefits and features, are available in the marketplace today to increase sample throughput. Such instrumentation has helped streamline the DNA extraction process for forensic laboratories. However, it is often highly specialized and expensive. Promega has developed the Maxwell™ 16 Instrument to offer laboratories an "easy landing" into the automation world and to supplement existing high-throughput instrumentation. The Maxwell™ 16 Instrument is compatible with the DNA IQ™ chemistry[a], which is a robust, reliable system to isolate a consistent amount of genomic DNA and is widely accepted both in manual and automated formats.

MAXWELL™ 16 INSTRUMENT

The Maxwell™ 16 Instrument provides high-quality automated DNA purification at a fraction of the cost of high-throughput instruments. This automated paramagnetic-particle handler uses predispensed, ready-to-use extraction reagents in disposable cartridges. To use the Maxwell™ 16 Instrument, scientists simply place samples directly into the reagent cartridge, place each cartridge into the Maxwell™ 16 Instrument and select "Run" from the navigation menu. Most forensic samples require a short preprocessing step prior to DNA extraction to remove solid support material that can interfere with instrument function. After the Maxwell™ 16 extraction process is complete, the purified DNA is ready for downstream applications, such as STR amplification and analysis.

The Maxwell™ 16 Instrument operates differently than many automated DNA purification instruments. Rather than moving liquids from one well to another to bind genomic DNA, wash away contaminants and elute purified DNA, the paramagnetic particles are moved from well to well during the purification process by individual magnets and disposable plungers. Between 1 and 16 samples can be processed during a single run, which requires approximately 15–20 minutes. Each reagent cartridge is dedicated to a specific sample during the extraction to help prevent sample cross-contamination.

Other attractive features of this instrument include the simplicity of installation and operation. There are no complicated installation procedures or automated methods to load and optimize; only two steps are involved: remove the instrument from the packaging, and plug it into a power outlet. The initial calibration is performed automatically each time the instrument is turned on, eliminating the laborious calibration procedures that are typical of more complicated instrumentation. Operating the instrument is simple through the use of a small navigation liquid-crystal display (LCD) (Figure 1, top panel). The small size and low cost provide laboratories with the flexibility to incorporate the Maxwell™ 16 Instrument into their existing processes rather than redevelop their workflow based on instrument requirements. As an approximately 1-foot cube, the instrument occupies little space (Figure 1, bottom panel).

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FORENSIC SAMPLE EXTRACTION

Prefilled Maxwell™ 16 reagent cartridges (Figure 2) allow scientists to continue to benefit from the DNA IQ™ chemistry and obtain consistent DNA concentrations and reliable performance in downstream STR analysis. Two DNA IQ™ reagent cartridges will be available: one for high-DNA-content samples used in reference or database applications (described in this article) and another for low-DNA-content samples used in casework analysis.

FTA®blood-stain cards and buccal swabs are two of the most prevalent high-DNA-content reference sample types used in the forensic community today. Here we describe development of the DNA IQ™ reference cartridge using FTA®cards, buccal swabs and liquid whole blood.

SAMPLE TYPE CONSIDERATIONS

Although genomic DNA yields obtained using the DNA IQ™ System are relatively consistent for a sample type, yields for different sample types can differ, even when the amount of DNA present exceeds the DNA-binding capacity of the DNA IQ™ Resin. The reason for this difference lies in the nature of the samples. Liquid blood and blood stains on FTA®cards are more complex than buccal swabs and contain other components, such as protein, that compete with DNA for binding to the resin, resulting in lower yield. Buccal swabs have relatively low amounts of these components, so there is less competition for DNA binding. Thus, yields with buccal swabs tend to be higher. However, not all buccal swabs will contain the same number of shed cells, depending on the buccal cell donor and how the swab is collected. This contributes to the broader yield range observed with buccal swabs compared to that of liquid blood or samples on FTA®cards.

When developing the Maxwell™ 16 Instrument for forensic use with reference samples, we optimized the DNA IQ™ reagent cartridges with buccal swabs, FTA®cards and liquid blood to achieve consistent yield and STR performance. We used DNA IQ™ Resin at a saturated level to optimize genomic DNA purification from buccal swabs, blood card punches and whole blood.

Figure 1. The Maxwell™ 16 Instrument and close-up of the navigation liquid-crystal display.

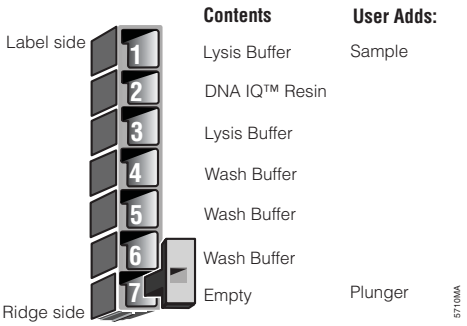

Figure 2. Contents of prefilled Maxwell™ 16 reagent cartridges.

MATERIALS AND METHODS

Cotton buccal swabs were collected from 16 individuals. Blood was drawn from four healthy adults. FTA®cards were stained with 125µl of fresh blood and allowed to dry overnight. Two 3mm

Table 1. Concentrations of DNA isolated from two 3mm FTA®blood-card punches, half of a cotton buccal swab and 20µl of whole blood.

Two 3mm FTA®Blood-Card Punches		One-Half of a Buccal Swab		Whole Blood (20µl)	
Sample Number	DNA Concentration (ng/µl)	Sample Number	DNA Concentration (ng/µl)	Sample Number	DNA Concentration (ng/µl)
1	0.29	1	1.32	1	0.73
2	0.31	2	1.28	2	0.82
3	0.29	3	2.20	3	1.09
4	0.33	4	1.00	4	1.06
5	0.38	5	1.23	5	0.35
6	0.37	6	1.31	6	0.43
7	0.40	7	2.11	7	0.48
8	0.41	8	2.07	8	0.55
9	0.25	9	1.81	9	0.30
10	0.29	10	1.91	10	0.40
11	0.33	11	1.88	11	0.37
12	0.28	12	1.91	12	0.34
13	0.31	13	2.01	13	0.41
14	0.38	14	2.29	14	0.40
15	0.41	15	1.75	15	0.35
16	0.32	16	1.73	16	0.39
Average	**0.33**	**Average**	**1.74**	**Average**	**0.53**

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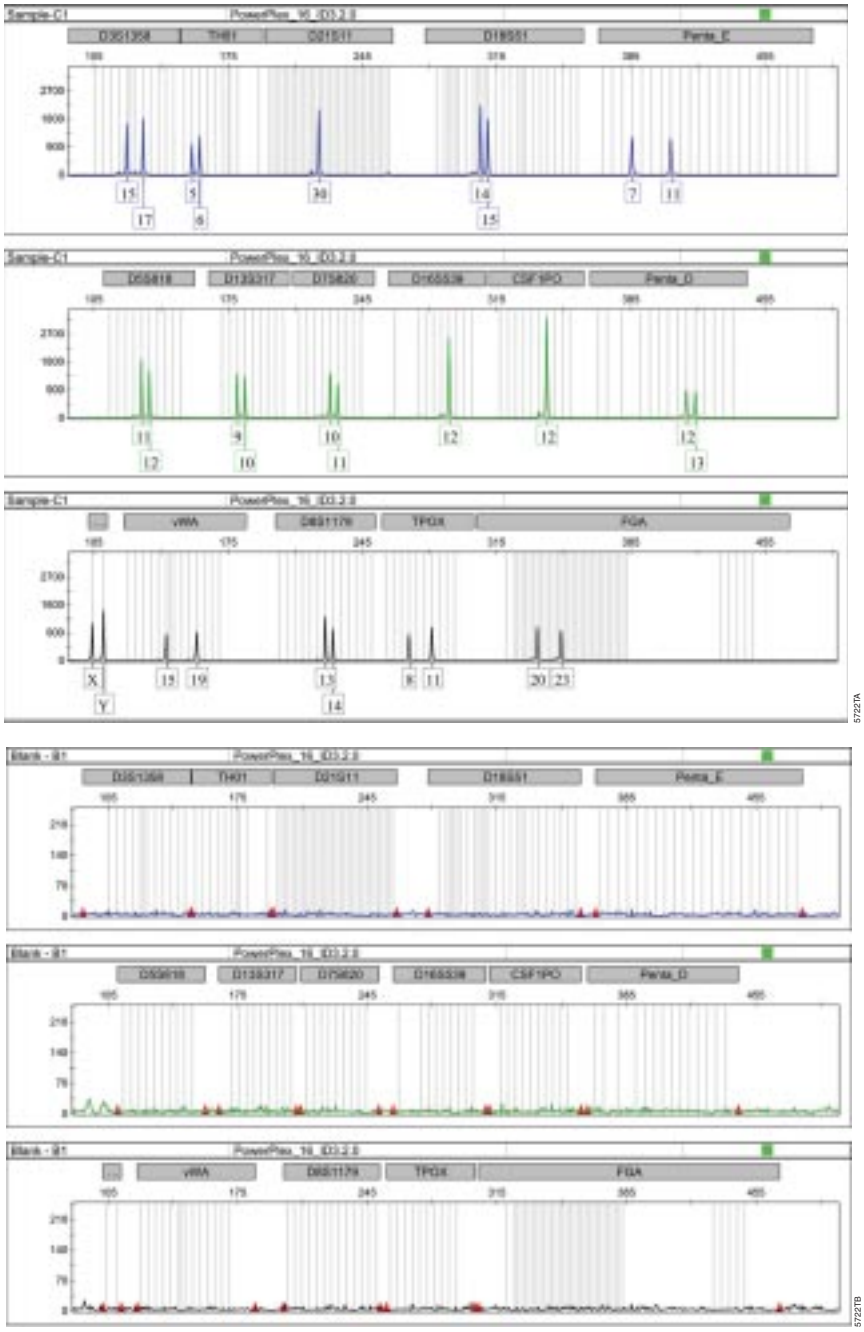

Figure 3. Absence of cross-contamination. DNA was isolated from buccal swabs (top panel) or blanks (bottom panel) using the Maxwell™ 16 Instrument. DNA was amplified for 32 cycles using the PowerPlex®16 System and a GeneAmp®PCR System 9600 thermal cycler. Amplified products were analyzed on an ABI PRISM®310 Genetic Analyzer.

punches were processed for each sample. To rehydrate and remove genomic DNA from the solid support, each swab or set of FTA®card punches was preprocessed with 500µl of DNA IQ™ Lysis Buffer containing 10mM DTT and incubated for 30 minutes at 70°C (swabs) or 30 minutes at 95°C (blood cards). The Lysis Buffer and swab or card punch were centrifuged through a DNA IQ™ Spin Basket at 14,000 × *g* for 2 minutes to separate the lysate from the solid support. Whole blood samples (20µl) were not preprocessed. Lysates and whole blood samples were processed with Maxwell™ 16 DNA IQ™ reagent cartridges. DNA was eluted in 300µl of DNA IQ™ Elution Buffer, and the DNA concentration was determined. The results are shown in (Table 1).

CROSS-CONTAMINATION

To evaluate sample-to-sample cross-contamination, DNA IQ™ cartridges containing buccal swabs or blank swabs were placed in the Maxwell™ 16 Instrument in an alternating pattern, and the Maxwell™ 16 method was run. Standard 25µl PowerPlex®16 System[b–d] reactions were set up to amplify 1µl of eluted DNA from buccal swabs or 10µl from blanks. Blank samples contained no amplification products, as expected, despite amplifying and analyzing ten times as much input material. High-quality STR profiles were obtained from reference samples tested. Representative profiles are shown in Figure 3.

CONCLUSIONS

We describe the development of an affordable, high-quality automated instrument for use with the DNA IQ™ System. This instrument offers scientists the flexibility to extract DNA from up to 16 samples in approximately 15–20 minutes after preprocessing. The Maxwell™ 16 Instrument and prefilled reagent cartridges provide consistent DNA yields from a variety of sample types without detectable cross-contamination between samples.