Robotic Extraction of Mock Sexual Assault Samples Using the Biomek® 2000 and the DNA IQ™ System

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INTRODUCTION

Forensic scientists are routinely faced with the challenge of isolating DNA from a large array of tissue and cell types. The variety of substrates upon which cellular material has been deposited, some of which may contain inhibitors of PCR, can make the process more difficult (1). Therefore, any robotic system applied to the extraction of forensic casework samples must be robust enough to address these variations. The Biomek® 2000 used in conjunction with the DNA IQ™ System[a] has proven to be an efficient robotic system designed to handle the challenges of routine casework samples.

The DNA IQ™ System uses silica-coated magnetic beads to separate DNA from cellular debris. Cells are lysed in a powerful lysis buffer, and the lysate is mixed with the magnetic beads. The beads saturate at approximately 100ng of bound DNA, and the excess DNA is removed by pipetting. Once bound to the magnetic resin, the DNA is pipetted and vigorously shaken several times in wash buffer, then eluted using heat. The Biomek® 2000 is equipped with a magnetic plate, a shaking platform and a thermal exchange unit to perform these necessary steps.

CONTAMINATION STUDIES

A number of exploratory and validation studies have been performed on the Biomek® 2000/ DNA IQ™ System to evaluate the viability of this automated system for use with forensic samples. Fundamental questions needed to be answered before moving ahead with extensive validation work. First, does the robotic, open-plate format cause contamination? To answer this question, two sample formats were used repeatedly, and extracted samples were analyzed to test

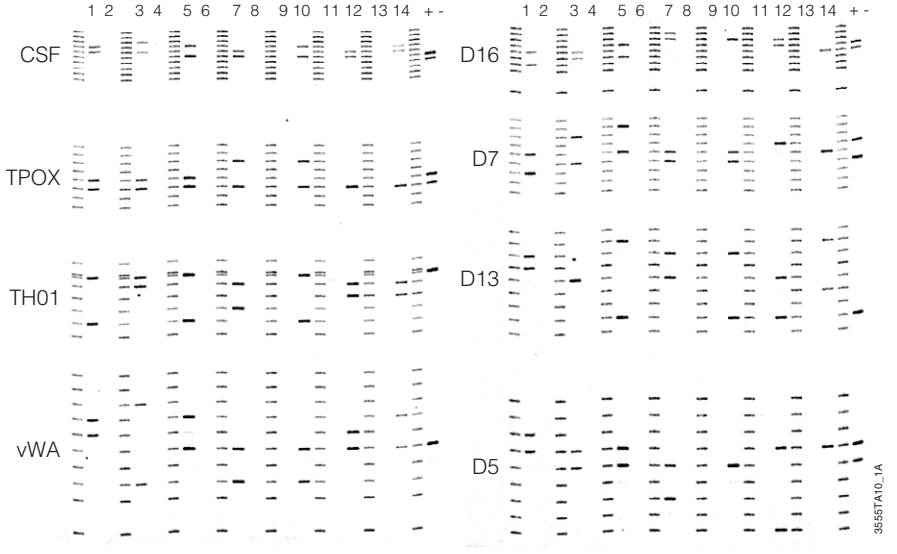

Figure 1. Amplified DNA samples using the PowerPlex® 1.1 System from the 88-sample checkerboard contamination study. Numbers 1–14 indicate sample numbers, "+" indicates positive control (K652), and "–" indicates negative control. Amplified DNA was analyzed by electrophoresis using a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® instrument).

for contamination. The first is the zebra-stripe format test: alternating columns of samples containing an abundant source of DNA with columns containing reagent blanks (8 sample wells per column). Therefore, a column of samples containing abundant DNA was processed adjacent to a column of reagent blanks in a striped pattern on the plate. The samples containing DNA were bloodstains cut into 5mm^2 squares. DNA was eluted from the magnetic beads into 100µl of sterile water, then quantified, amplified, and typed using the PowerPlex® 1.1 System[b,c] (2). The first two trials of this test detected some contamination. The software method used was modified to accommodate sample loading into a 96 deep-well plate in place of the more shallow Greiner plate and to remove an initial shaking step. A subsequent zebra stripe experiment showed no contamination with the 40 samples that were isolated.

The second contamination test is a checkerboard sample format: samples containing abundant DNA were alternated with reagent blanks in a checkerboard pattern across a 96 deep-well plate (Figure 1). All 128 samples (88 sample and 40 sample methods) tested negative for any detectable contamination.

MOCK SEXUAL ASSAULT SAMPLES

Sexual assault cases frequently constitute the majority of DNA cases received by a forensic laboratory. Presently, no robotic system is available that can separate sperm from non-sperm cells and thus perform a differential extraction (3) from start to finish. However, the first step of separating fractions can be performed manually. Subsequently, the DNA from E-cell (epithelial or non-sperm cell) lysates and sperm pellets can be extracted robotically, saving analysts a substantial amount of time. Any robotic extraction of sexual assault samples must at least be able to generate sample DNA, equivalent in both quality and yield to that generated by manual DNA extraction methods. Therefore, a thorough examination of the Biomek® 2000/DNA IQ™ System's ability to isolate DNA from sexual assault samples needed to be performed. The first step was to ascertain whether sperm cells could be successfully lysed and the DNA purified by the robotic system. Mock sexual assault samples were prepared using previously donated vaginal swabs and semen from a known donor, which was deposited onto sterile cotton swabs in 1:2 and 1:4 dilutions. The E-cells were lysed manually, the sperm cells pelleted, and a portion of the lysates and the entire sperm pellets were loaded onto the Biomek® 2000 for DNA extraction. DNA was eluted off the magnetic beads into 100µl of sterile water. High-quality DNA was obtained and typed accurately using the PowerPlex® 1.1 System (data not shown).

Once it was demonstrated that the Biomek® 2000/DNA IQ™ System could successfully complete the differential extraction process with the E-cell lysates and intact sperm, the next question addressed was whether this automated system could produce DNA of comparable quality and yield to that produced by manual extraction. A comparative study was designed to measure the performance of the Biomek® 2000/DNA IQ™ System with respect to manual extraction of similar if not identical samples. Samples were prepared in the following manner:

1. Sets of vaginal swabs from 5 different donors were selected.
2. Duplicate mock sexual assault swabs were prepared using semen from a single donor at the following dilutions: 1:10, 1:100, 1:1,000 and 1:10,000 for three sets, 1:10, 1:100, 1:200 and 1:400 for one set and 1:100, 1:200, 1:400 and 1:800 for the last set.
3. Once dried, the swabs were cut into 1/2, 1/4 and 1/8 portions.
4. The E-cells were lysed and the sperm cells pelleted and washed.
5. The samples were split evenly with one half going to an analyst to complete the extraction manually and the other loaded onto the Biomek® 2000 for a robotic DNA extraction.

Yields and quality of the DNA from the sperm fractions processed by the Biomek® 2000/DNA IQ™ System were comparable and frequently superior to those obtained by manual extraction (Figure 2). In less experienced hands, the Biomek® 2000/DNA IQ™ System clearly outperformed the manual extraction (data not shown). Results obtained by experienced users were equivalent to those achieved with the robot. Therefore, the Biomek® 2000/DNA IQ™ System is not only capable of outperforming its human counterpart, but it also delivers a more consistent product.

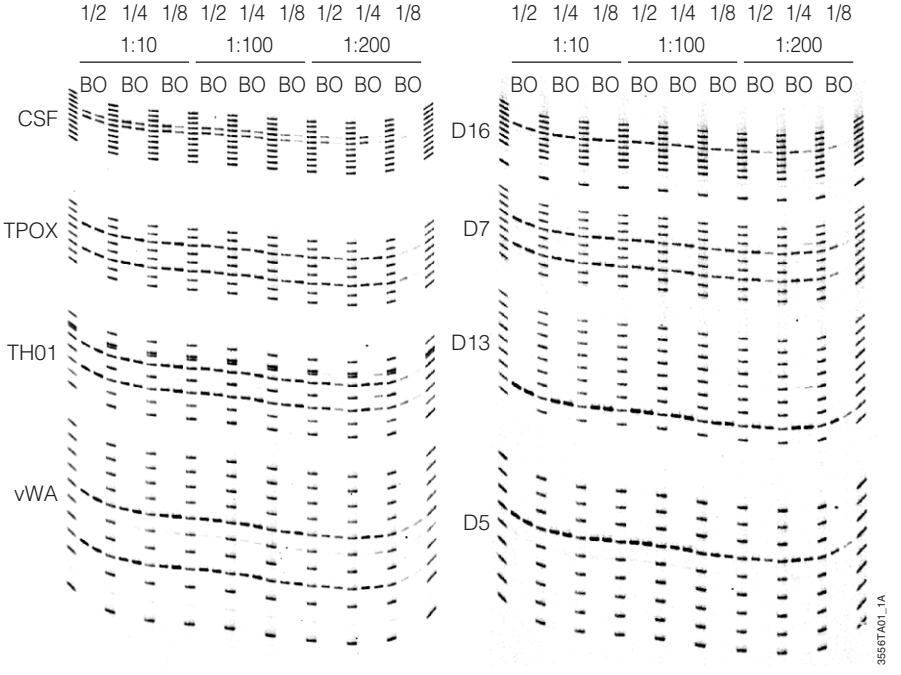

Figure 2. PowerPlex® 1.1 mock sexual assault comparison study. Duplicate samples were extracted manually (indicated by an "O" above the lane, for organic) or robotically (indicated by a "B" above the lane, for Biomek® 2000). Semen dilutions (1:10, 1:100 and 1:200) for each set of swabs are indicated above the corresponding six sample lanes, which contain DNA extracted from the indicated swab portion (1/2, 1/4 or 1/8). Amplified DNA was separated by electrophoresis in a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585nm scan (left panel) and the 505nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO® instrument).

The maximum sample volume for use with the 96 deep-well plate is limited to 100µl. Sperm cells are typically in a pellet of 50µl and therefore unaffected by the volume limit. Since the E-cell lysate is usually in a volume of 500µl, it was important that the yields from 100µl of E-cell lysate from the 1/2, 1/4 and 1/8 swab portions be sufficient for all DNA typing needs. Total yields of E-cell DNA extracted on the robot were calculated (Figure 3), and sufficient E-cell DNA could be obtained using robotic extraction methods. In fact, 1/8 swab portions provided more than enough DNA for an E-cell DNA profile.

The amount of time saved by using the Biomek® 2000/DNA IQ™ System to extract the forensic samples can be substantial. The time it takes to complete the organic extraction manually for a single sample is 5 hours and 5 minutes (after E-cells have been lysed, and sperm cells pelleted and washed). Of course, additional samples will lengthen the amount of time proportionately. In comparison, the robot takes 1 hour and 15 minutes to extract the DNA from 40 samples and 1 hour and 50 minutes to extract 88 samples. Therefore, the absolute minimum amount of time saved is 3 hours and 50 minutes or half of a day.

EXTRACTION OF OTHER CELL AND TISSUE TYPES

Since a variety of cells and tissue types are encountered in routine forensic casework, the Biomek® 2000/DNA IQ™ System was evaluated to determine its capability to isolate DNA from a variety of sources. Dried bloodstains, E-cell lysates, intact sperm cells, muscle, heart, brain, liver and buccal swabs were extracted using the Biomek® 2000/DNA IQ™ System and successfully typed using the PowerPlex® 1.1 System (data not shown).

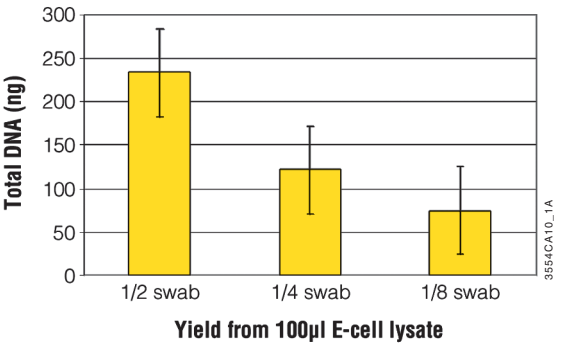

Figure 3. Bar graph depicting the total yield of E-cell DNA generated from extraction on the Biomek® 2000 robot using 100µl of lysate. Yields were determined by measuring DNA concentration using the QuantiBlot® kit.

CONCLUSION

A completely automated system for extraction of sexual assault samples is currently not available. However, once the E-cells have been separated from the sperm cells, robotic DNA extraction using the Biomek® 2000/DNA IQ™ System can be accomplished. Moreover, when sperm DNA is limited, the Biomek® 2000/DNA IQ™ System generates DNA of similar, and sometimes better, quality and yield than that obtained by manual extraction of a duplicate sample. Because of the adaptability of the Biomek® 2000/ DNA IQ™ System, this instrument has the potential to handle future applications of emerging cell separation technologies. It may be possible on a robotic platform, to separate sperm cells from non-sperm cells with the use of an anti-sperm antibody conjugated to a magnetic bead. One can envision a completely automated system where both cell separation and DNA extraction are performed on the same robot. The Biomek® 2000/DNA IQ™ System may be uniquely poised to proceed with that application when the technology becomes available.

The time saved when compared with manual extraction, as well as the ability to extract a variety of tissue and cell types, makes the Biomek® 2000/DNA IQ™ System attractive for casework applications. Further validation work on the Biomek® 2000/DNA IQ™ System must be performed in order to complete our evaluation and validation prior to its application for forensic casework. Although no contamination of the samples was detected after modifying the method and changing the format, special circumstances might require the use of manual extraction methods, for example, when an evidentiary sample may be completely consumed due to limited available material.

ACKNOWLEDGMENTS

We would like to acknowledge the participation of other members of the Virginia Division of Forensic Science: Beth Ballard, Missy Baisden, Brian Covington, Shelley Smith and Colleen Young. We would also like to thank Allan Tereba and Dan Kephart at Promega Corporation for all their hard work at making the technology and this study possible.

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