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 the 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 may be such a robotic system designed to handle the challenges of routine casework samples.

The DNA IQ System utilizes silica coated magnetic beads to separate the DNA from the cellular debris. Cells are lysed in a powerful lysis buffer and the lysate mixed with the magnetic beads. The beads saturate at approximately 100ng of bound DNA and the excess DNA is pipetted off. Once bound to the magnetic resin, the DNA is pipetted and vigorously shaken several times in wash buffer, then eluted off the beads with heat. The BioMek® 2000 has been equipped with a magnetic plate, shaking platform and 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 combined system for use with forensic samples. Fundamental questions needed to be both asked and answered before moving ahead with extensive validation work: First, is the robotic, open plate format susceptible to contamination? To answer this question, two sample formats were employed repeatedly and extracted samples analyzed in order to detect any contamination. The zebra stripe format test alternated 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 would be processed adjacent to a column of reagent blanks in a striped pattern on the plate. The samples containing the abundant source of DNA were bloodstains cut into 5 mm^2 squares. All DNAs were eluted from the magnetic beads into 100µl of sterile DiH_2O, quantified, amplified, and typed for the PowerPlex™ 1.1 loci (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 removal of an initial shaking step. A subsequent zebra stripe experiment showed no contamination for the 40 samples that were isolated. The second contamination test employed a checkerboard sample format, again using the 96 deep well plate. Samples containing an abundant source of DNA were alternated with reagent blanks in a checkerboard pattern across the 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 comprise the majority of DNA cases that a forensic laboratory may receive. Presently, no robotic system is available which can separate sperm from non-sperm cells and thus perform a differential extraction (3) from start to finish. However, the first step of separating the sperm and the non-sperm fractions can be performed manually. Subsequently, the DNA from E-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 a minimum, be able to

generate sample DNA, equivalent in both quality and yield to that generated by manual DNA extraction methods. Therefore we felt that 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 the vaginal swabs in neat, 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. All DNAs were eluted off the magnetic beads into 100 ul of sterile DiH_2O. High quality DNA was obtained and typed accurately for all samples at the PowerPlex™ 1.1 system loci (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 the BioMek® 2000/DNA IQ™ System could function as well as the manual extraction process. To be useful for the extraction of casework samples, a robotic system must 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 very 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, 1:1000 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 in into ½, ¼, 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 half 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 that obtained by manual extraction (Figure 2). In less experienced hands, the BioMek® 2000/DNA IQ™ System clearly outperformed the manual extraction (data not shown), however, the performance of more seasoned scientists was more equivalent to the robot. Therefore, the BioMek® 2000/DNA IQ™ System is not only capable of sometimes out-performing its human counterpart, but it also delivers a more consistent product.

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 thus are unaffected by the volume limit. Since the E-cell lysate is 500µl, it was important that the yields from 100µl of E-cell lysate from ½, ¼ and 1/8 a swab would be sufficient for all DNA typing needs. Total yields of E-cell DNA extracted on the robot were calculated (Figure 3) and it was determined that sufficient E-cell DNA could be obtained. In fact, 1/8 swab 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. Consider the point at which the E-cells have been lysed and the sperm cells pelleted and washed three times as time zero and the end point is when the DNA is ready for quantitation. The time it takes to complete the organic extraction manually for a **single** sample is 5 hours and 5 minutes. 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 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 system was evaluated to determine its capability of isolating DNA from a wide 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 for the PowerPlex™ 1.1 loci (data not shown).

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 generated as high a yield and DNA quality (and sometimes higher), than the manual extraction for the 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 application to casework. Further validation work on the BioMek® 2000/DNA IQ™ System must be performed in order to complete our evaluation and validation prior to the application to forensic casework. Although no contamination of the samples was detected after modifying the method and changing the format, it would behoove the forensic scientist to employ caution. For example, when an evidentiary sample may be completely consumed due to limited material available, it would be a wise to extract that sample manually.

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FIGURE LEGENDS

Figure 1. PowerPlex™ 1.1 gel containing amplified DNA samples from the checkerboard contamination study. Numbers 1-14 indicate sample numbers. + = postive control (K652), - = negative control. Amplified DNA was electrophoresed in a 6% polyacrylamide gel (Gibco BRL)

for 2 hours at 50 watts. Both the 585 nm scan (left panel) and the 505 nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO™).

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, ¼ or 1/8). Arrows point to loci or alleles which have dropped out or are greatly reduced in signal. Amplified DNA was electrophoresed in a 6% polyacrylamide gel (Gibco BRL) for 2 hours at 50 watts. Both the 585 nm scan (left panel) and the 505 nm scan (right panel) are shown (gel imaging performed using a Hitachi FMBIO™).

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 (Applied Biosystems).