B95 Cost Effective Protocol for Automated Buccal Specimen Extraction Using DNA IQ™ Resin on the Biomek® 2000

Chris Macaraeg, BS, Pace University, 1 Pace Plaza, New York, NY 10038; and Ewelina J. Bajda, BS, Mechthild Prinz, PhD, and Theresa A. Caragine, PhD, Office of the Chief Medical Examiner of the City of New York, 520 First Avenue, New York, NY 10016*

After attending this presentation, attendees will learn cost effective, automated exemplar extraction using DNA IQ (Promega) resin with yields consistently sufficient for direct amplification and without low level DNA contamination.

This presentation will impact the forensic community and/or humanity by demonstrating a cost effective protocol for automated buccal specimen extraction using DNA IQ (Promega) resin on the Biomek 2000 with yields consistently sufficient for direct amplification and without low level DNA contamination.

This presentation will demonstrate a cost effective protocol for auto- mated buccal specimen extraction using DNA IQ™ (Promega) resin with yields consistently sufficient for direct amplification and without low level DNA contamination.

According to the manufacturer's recommended protocols, the use of the DNA IQ™ kit provides a consistent recovery of DNA so that quanti- tation is unnecessary prior to amplification. The kit has also shown to be easily implemented for automation on the Biomek® 2000 (Beckman Coulter). However, laboratory studies demonstrated that the yield varied from tissue and blood samples; moreover, low-level contamination was found. In order to resolve these issues for exemplar extraction, the authors optimized the protocol and redesigned the layout as well as robotic program for the Biomek® 2000.

In order to use Promega's DNA IQ kits more efficiently, the resin was titrated to 0.5 µL with the lowest amounts of the resin tested for point of saturation. One microliter gave similar results to the recommended seven microliters for up to 100 ng of DNA, even though it was found that only fifty percent of the input DNA was recovered for both of the conditions. Although using the suggested volume of resin did produce a greater yield for 150 ng than lower amounts of resin, this amount far exceeds the required amount of template DNA for amplification; therefore, the use of 1 ul of resin is sufficient for exemplar typing.

The adjustments in sample size, digest and elution volume were made in order to deliver uniform DNA yields. The decisions were formulated based on the quality and the completeness of the profiles generated on the ABI's Prism 3100 Genetic Analyzer using 1-kV and 22-second injection parameters and 2 µL of the sample amplified using AmpFISTR Identifiler PCR Amplification Kit (ABI) for 28 cycles. High Sensitivity amplification protocol was used, which includes a two-minute annealing temperature and half reaction volume. Using 50 µL of the digest from half of a swab and 100 µl of its elutant produced full profiles. Some peaks from a few samples were below threshold (75 RFU); nevertheless, with the application of more voltage (3kV) these peaks were resolved. Alternatively, more amplification product could have been injected.

Previous studies with the DNA IQ recommended protocol on the Biomek 2000 demonstrated low level contamination in checkerboard pattern with alternating negative wells. Similarly, the use of seal with the Biomek 2000's current configuration precluded tip touch, which ulti- mately led to increased occurrence of spurious alleles. Although it may be impossible to eliminate all sources of contamination, several aspects can be addressed such as aerosols created by shaking, and dripping during DNA transfer, for example when excess DNA is moved to the waste reservoir from the sample and resin mixture. Therefore, the pipetting as well as shaker parameters were modified, and the layout of the Biomek was rearranged. These modifications permit a 96-well plate to be used in its entirety for sample extraction without the need for alternating blanks, as no alleles were determined with the High Sensitivity 31-cycle amplification.

Therefore, 50 ul of a buccal specimen digest with 1 uL of resin and 100 ul of the resultant elutant generate robust, reliable profiles. With a minor modification in the digest volume, this program can be applied to bloodstain exemplars. In this manner, the developed methodology is cost effective and significantly improves laboratory throughput without com- promising sample quality.

DNA Extraction, Automation, Buccal Swabs

 ** Presenting Author*