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Automating the DNA IQ™ System on the Biomek® 3000 Laboratory Automation Workstation

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INTRODUCTION

Over the past four years the Beckman Coulter Biomek®2000 Laboratory Automation Workstation has been used successfully in many forensic labs to automate DNA extraction from casework and database samples using the Promega DNA IQ™ System[a]. Recently Beckman Coulter has released the Biomek®3000, which provides additional functionality not found in the Biomek®2000, such as sample tracking and the ability to create a single method with variables to run different numbers of samples and sample types.

Promega has developed a method for isolating DNA samples on the Biomek®3000 using the DNA IQ™ System (Cat.# DC6700). The method uses a new electrical heater and shaker instead of the previously used thermal exchange unit heated with recirculating water and the Micromix®5 shaker. In addition, the Greiner U-bottom plate that was previously used with the Biomek®2000 method has been replaced with a polypropylene 1.2ml Round-Bottom Deep Well Plate (Cat.# V6771). This allows more vigorous shaking during the wash and elution steps without risk of solutions splashing out of the wells and contaminating adjacent wells. To facilitate heating of this deep-well plate during the elution step, we have also developed the new Deep Well Heat Transfer Block.

The single method allows the user to choose between “aqueous” and “swab” sample types and designate the number of samples being processed. Aqueous sample types are typically those that are preprocessed by incubating with proteinase K prior to extraction on the Biomek®3000, whereas “swab” samples refer to DNA on a solid support (e.g., buccal swab or FTA®card punch) that are preprocessed by incubating in DNA IQ™ Lysis Buffer in a Slicprep™ 96 Device (Cat.# V1391), followed by centrifugation to separate the lysate from the solid support (1).

The Biomek® 3000 method for the DNA IQ™ System gives consistent DNA yields with no detectable cross-contamination between samples as judged by PowerPlex® 16 analysis.

NEW HARDWARE

Promega has integrated four new pieces of hardware on the Biomek®3000 workstation: the V&P Scientific Heating Block (Cat.# V6761), Deep Well Heat Transfer Block (Cat.# V6741), VARIOMAG®Teleshake shaker (Cat.# V6751) and Shaker Integration Plate (Cat.# V3691) for holding the shaker on the deck of the Biomek®3000 (Figure 1). The new V&P Scientific Heating Block achieves a higher elution temperature, leading to more efficient elution, and eliminates the need for a recirculating water bath with the potential risks associated with leaking. The new VARIOMAG®Teleshake shaker, in combination with a 1.2ml Round-Bottom, Deep Well Plate, allows more vigorous shaking of the DNA IQ™ Resin during the method without risk of splashing. These new hardware components are available from Promega.

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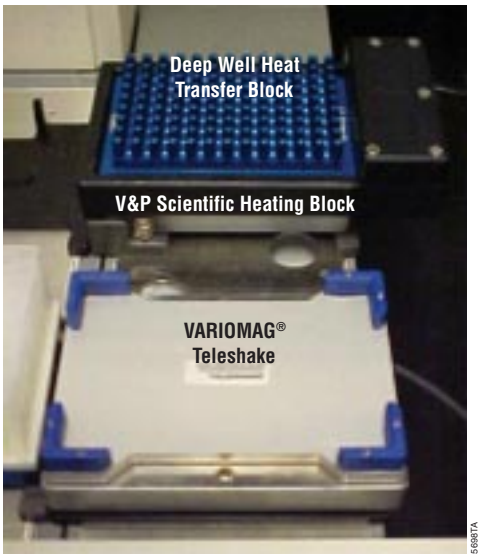

Figure 1. New integration hardware for the DNA IQ™ method on the Biomek®3000. The new Deep Well Heat Transfer Block is placed on top of the V&P Scientific Heating Block at position A6 on the deck of the Biomek®3000. The VARIOMAG®Teleshake is placed at position B6 and held in place by the Shaker Integration Plate (not visible in this picture).

HEATING PROFILE WITH NEW HEATER AND DEEP WELL HEAT TRANSFER BLOCK

To determine the efficiency of heat transfer during elution, we measured the temperature of 100µl of DNA IQ™ Elution Buffer in three separate wells of a 1.2ml Round-Bottom, Deep Well Plate on the Deep Well Heat Transfer Block with the V&P Scientific Heating Block set at 80°C or 85°C. Elution of DNA from the DNA IQ™ Resin is most efficient at temperatures greater than 65°C. The data are shown in Tables 1 and 2.

With the V&P Scientific Heating Block set at 85°C, the temperature of the elution buffer reached at least 65°C during the elution step. Wells at the edge of the plate were at a comparable temperature to those in the middle. Based on these results, we decided to set the V&P Scientific Heating Block at 85°C for elution during the Biomek®3000 method.

Table 1. Temperature in Wells of a 1.2ml Round-Bottom Deep Well Plate with the 80°C Heat Setting of the V&P Scientific Heating Block (°C).

Well	Baseline	Heat for 2.5 minutes	Shake for 30 seconds	Heat for 2.5 minutes	Shake for 30 seconds
H1	21	62	59	69	61
E6	21	61	58	70	65
D12	21	61	58	70	64
Mean	21	61	58	70	63

Table 2. Temperature in Wells of a 1.2ml Round-Bottom Deep Well Plate with the 85°C Heat Setting of the V&P Scientific Heating Block (°C).

Well	Baseline	Heat for 2.5 minutes	Shake for 30 seconds	Heat for 2.5 minutes	Shake for 30 seconds
H1	21	64	59	71	66
E6	21	62	61	72	68
D12	21	63	61	71	68
Mean	21	63	60	71	67

CONTAMINATION STUDIES

To evaluate cross-contamination with the Biomek®3000 method, cotton buccal swabs and DNA IQ™ Lysis Buffer blanks were processed in a checkerboard pattern in a Slicprep™ 96 Device. Each well of the Slicprep™ 96 Device received 400µl of DNA IQ™ Lysis Buffer. The top of the 96 Spin Basket was sealed with a foil sealer, and the whole Slicprep™ 96 Device was heated at 70°C for 1 hour. Following heating, the Slicprep™ 96 Device was centrifuged at 1,500 × *g* for 5 minutes to separate the "swab lysate" from the buccal swab. Following removal of the 96 Spin Basket containing the spent buccal swabs, the 2ml 96-well, deep-well plate containing the "swab lysate" was placed on the Biomek®3000 deck at position B4 and the DNA IQ™ method for "swab" samples initiated. Samples were eluted from the DNA IQ™ Resin in 100µl of DNA IQ™ Elution Buffer. The swab samples (1µl) and blank samples (10µl) were amplified in a standard 25µl PowerPlex®16 System[b–d] reaction. Representative results for swab and blank samples are shown in Figure 2. Amplification of DNA from buccal swabs resulted in strong signals. There were no detectable amplification products in reactions with blank samples.

CONSISTENCY OF DNA YIELDS

To investigate the consistency of DNA yields obtained with the new Biomek® 3000 method, we isolated DNA from 12 × 20µl liquid blood samples from one individual (to minimize variability in yield due to variations in the blood). Blood samples were initially pretreated at 56°C for 1 hour with 80µl of the proteinase K solution described in the *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* #TB307). The 2.2ml 96-well, deep-well plate containing the samples was placed on the Biomek® 3000 deck, and the "aqueous" sample method was initiated. Samples were eluted with 100µl of DNA IQ™ Elution Buffer, and yields were determined using the Quant-iT™ PicoGreen®dsDNA reagent. The average yield was 0.93 ± 0.13ng/µl, demonstrating that DNA yields were consistent for the Biomek®3000 method when processing samples with DNA in excess of the binding capacity of the DNA IQ™ Resin.

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YIELD VERSUS INPUT DNA

Two classic features of the DNA IQ™ System are the high efficiency of recovery at low levels of DNA and recovery of a relatively fixed amount of DNA as the amount of DNA in the sample increases. To ensure that this is true of the DNA IQ™ method on the Biomek®3000 workstation, DNA was isolated from increasing volumes (0.05–20µl) of liquid whole blood isolated from one individual.

Figure 3 shows the results obtained. The results show the classic linear response of increasing recovery with increasing input DNA at low levels of DNA and then a plateau as the DNA starts to saturate the resin (Figure 3). Recovery rates at 0.05µl of blood were in the 80–90% range.

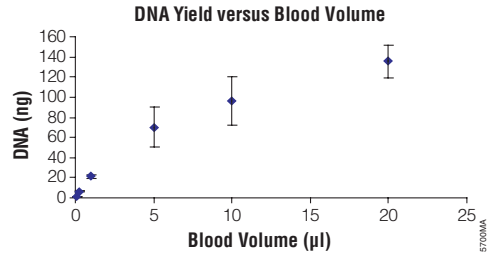

Figure 3. Characteristic yield versus input profile for the DNA IQ™ System. Four replicates of liquid blood samples, ranging from 0.05µl to 20µl, were digested with proteinase K at 56°C for 1 hour, then DNA was extracted using the DNA IQ™ System and the Biomek®3000 workstation. DNA yields were determined by quantitation with the Quant-iT™ PicoGreen®dsDNA reagent.

CONCLUSIONS

In conjunction with the Deep Well Heat Transfer Block, the new V&P Scientific Heating Block provided efficient heat transfer to samples during elution in a 1.2ml Round-Bottom Deep Well Plate. The Biomek®3000 method for the DNA IQ™ System gave consistent DNA yields from samples where DNA content exceeded the binding capacity of the resin and efficiently isolated DNA from samples containing trace amounts of DNA.

When isolating DNA from buccal swabs using the Slicprep™ 96 Device and the Biomek®3000 method, there was no detectable cross-contamination between samples, as judged by PowerPlex®16 analysis of a checkerboard of swabs and blanks, even when ten times more blank material than swab material was amplified.

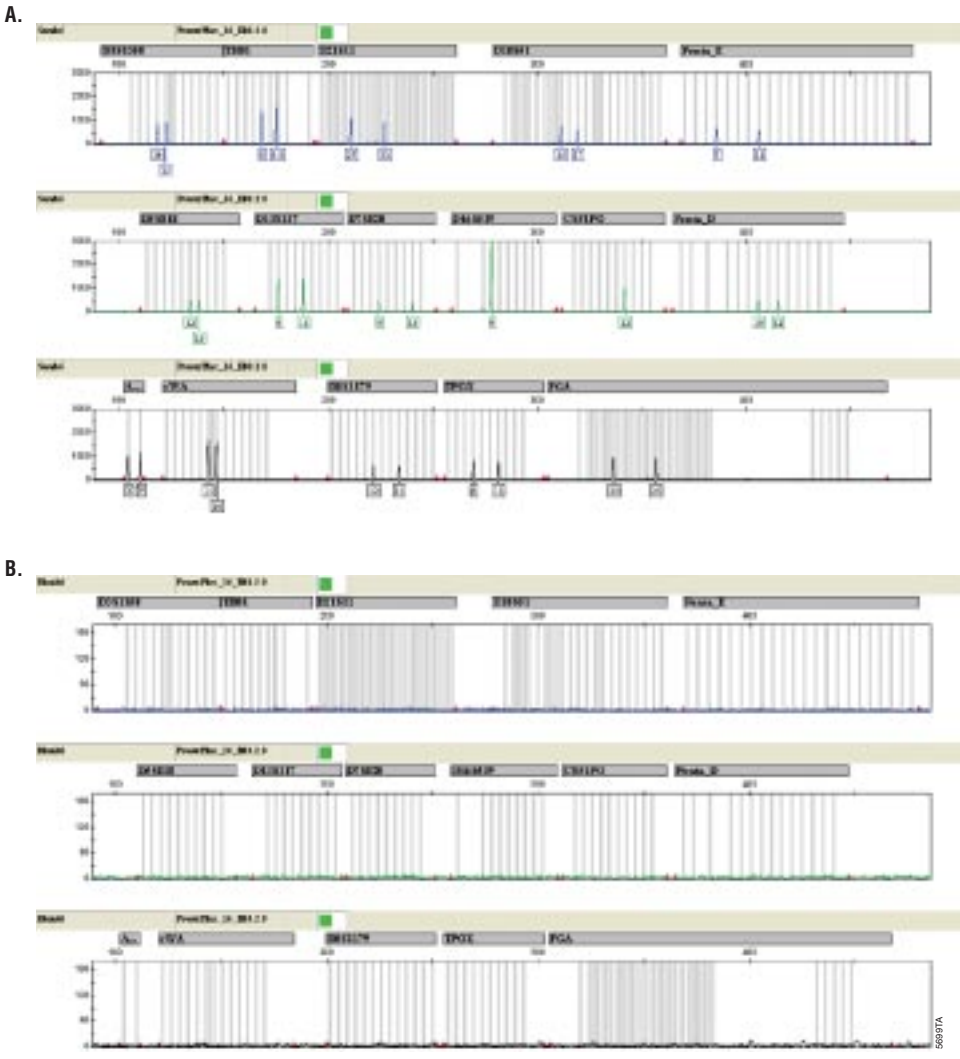

Figure 2. Absence of cross-contamination. Cotton buccal swabs and blanks were preprocessed in a checkerboard pattern in a Slicprep™ 96 Device and DNA subsequently isolated using the DNA IQ™ System on the Biomek®3000 workstation. Samples were amplified using the PowerPlex®16 System for 32 cycles on a GeneAmp®PCR System 9700 thermal cycler and analyzed with an ABI PRISM® 3130 Genetic Analyzer. Ten times more material from the blank sample than material from the swab sample was amplified. **Panel A.** A representative profile from a buccal swab. **Panel B.** A representative profile from a blank sample.

REFERENCES

1. Tereba, A. *et al.* (2005) High-throughput processing of samples on solid supports using the Slicprep™ 96 Device. *Profiles in DNA* **8**(2), 3–5.