Optimization of a DNA Extraction Protocol for the Beckman Coulter Biomek® NXP Laboratory Automation Workstation

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ABSTRACT

Increasing demands for DNA testing have resulted in severe DNA backlogs across the country. In response to these backlogs, automation of processes in the DNA workflow has increased the rate at which these backlogs, along with incoming samples, are analyzed. For this purpose, Promega® has developed a protocol using DNA IQ™ on the Biomek® NXP for DNA extraction and purification. However prior to this extraction, samples must undergo a manual digestion process. This study sought to identify the optimal instrument settings, sample incubation conditions, and assess the risk of contamination. A modified protocol was developed that incorporated optimized incubation conditions and a spin basket to improve DNA yield. The elution volume setting was changed to adjust for downstream processes. However, cross-contamination remains a cause for concern.

INTRODUCTION

The Promega® DNA IQ™ system on the Biomek® NXP extracts and purifies DNA using deep well plates, allowing 96 samples to be extracted per run. This would increase sample throughput from that of manual extraction methods and significantly decrease the hands-on time required by analysts. However, sample solubilization must be performed offline prior to robotic handling. This step along with certain parts of the automated protocol must be optimized to maximize DNA yield and ease of use while minimizing the possibility of contamination.

A protocol was developed prior to this testing concordant with the validated Qiagen® M48 currently used for automated extraction. All modifications were compared to the original to ensure DNA yield was comparable.

MATERIALS

Sample Preparation: Hemacare BioResearch® Whole Blood, Dynarex sterile cotton tipped applicators

Extraction: DNA IQ™ on the Biomek® NXP
Quantification: Quantifiler® Trio, Applied Biosystems® 7500 Real Time PCR System

Amplification: Promega® Powerplex® Fusion, Applied Biosystems® GeneAmp PCR System 9700

Capillary Electrophoresis: Promega® Powerplex® Fusion,

Analysis: SoftGenetics® GeneMarker® HID software v2.7.1

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METHODS

Sample Preparation:

Whole blood or a dilution of whole blood was pipetted onto a sterile cotton swab.

Original Sample Digestion Protocol:

A digestion buffer consisting of 0.05% SDS and proteinase K was added to the substrate. This was incubated on a Thermomixer® at 56°C for 30 minutes, 99°C for 10 minutes, and 4°C for 10 minutes. The substrate was then transferred to a spin basket and centrifuged to remove additional lysate.

Elution Volume Study:

Digestion buffer was run through the DNA IQ™ extraction protocol on the Biomek NXP with the elution volume set to 37 µL. Samples were measured with a pipette for a rough estimate of actual elution volume.

Volume Reduction Tip Study:

The volume of the digestion buffer was doubled and used to solubilize a 1:10 dilution of whole blood. This was divided in half. One half was run using p200 tips to transfer the sample in the volume reduction step. One half was run using p1000 tips for this step. The samples were quantified, and DNA yield was compared.

Incubation Comparison Studies:

The digestion was performed with and without the 99°C and 4°C steps to determine if they were necessary to inactivate proteinase K prior to purification.* This was repeated while extending the 56°C incubation to 1 hour.

Spin Basket Comparison Study:

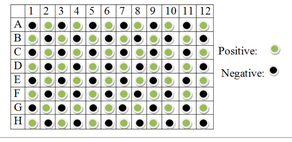
The digestion was performed on samples in Qiagen® Investigator® Lyse&Spin baskets as well as DNA IQ™ spin baskets. The Lyse&Spin baskets were incubated at 65°C to ensure the sample reached 56°C within the spin basket. The results were quantified and compared.

Modified and Original Protocol Comparison Study:

Samples were run on the original protocol and a new protocol incorporating a 1 hour 65°C incubation and the Qiagen® Investigator® Lyse&Spin baskets.

Cross-Contamination Study

A full plate of samples was solubilized using the digestion protocol, 48 of which were positive blood samples and 48 of which were negative buffer samples. These were placed in a checkerboard pattern to assess the possibility of cross-contamination.



*Results not included in poster.

RESULTS

Elution Volume Study

The average elution volume when the Biomek® NXP software was set to elute at 37 µL was 28.5 µL with a standard deviation of 1.30 µL.

Volume Redicution Tip Study

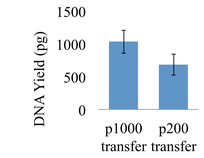


Figure 1. Comparison of DNA yields from extractions using p200 or p1000 tips in the volume reduction step of the protocol for DNA IQ™ on the Biomek® NXP.

Incubation Comparison Study

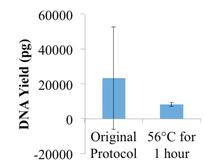


Figure 2. Comparison of DNA yield from a 1:5 dilution of whole blood using previous incubation protocol and a modified incubation protocol.

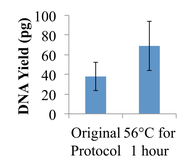


Figure 3. Comparison of DNA yield from a 1:100 dilution of whole blood using previous incubation protocol and a modified incubation protocol.

Spin Basket Comparison Study

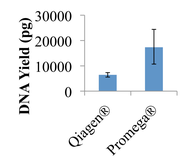


Figure 4. Comparison of DNA yield from a 1:5 dilution of whole blood using Qiagen® Lyse&Spin Baskets and Promega® DNA IQ™.

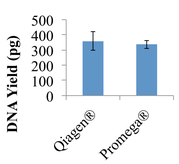


Figure 5. Comparison of DNA yield from a 1:100 dilution of whole blood using Qiagen® Lyse&Spin Baskets and Promega® DNA IQ™.

Modified and Original Protocol Comparison Study

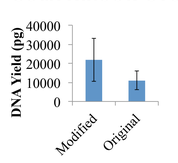


Figure 6. Comparison of DNA yield from a 1:5 dilution of whole blood using the modified and original digestion protocols.

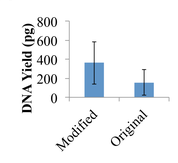


Figure 7. Comparison of DNA yield from a 1:100 dilution of whole blood using the modified and original digestion protocols.

Cross-Contamination Study

Analysis with Quantifiler® Trio identified 3 wells that should be negative with DNA in excess of this limit. These samples were amplified with Powerplex® Fusion for identification. Two wells contained alleles that could have come from the positive control. A third well contained an unknown profile not consistent with the positive control or lab personnel.

DISCUSSION AND CONCLUSIONS

Following the elution study, it was determined that 37 µL was an acceptable setting to elute within the range that is optimal for downstream processes. Additionally, the p200 tips were replaced with p1000 tips in the volume reduction step of the DNA IQ™ protocol on the Biomek® NXP, as their use yielded significantly more DNA. Beyond this, aspiration steps and tip touches were added to the instrument protocol. Adjustments were also made to the orbital shaker to attempt to account for expansion of deep well plates when heated. These could prevent the cross contamination observed in the cross-contamination study, if it occurred on the instrument. Future studies should evaluate the possibility of contamination during the digestion, including the removal of consumables that may have contributed the unknown profile.

The initial removal of the 99°C and 4°C incubation periods in the digestion protocol showed a significantly lower DNA yield at a 1:100 dilution of whole blood. The extension of the 56°C incubation period to 1 hour showed comparable DNA yields at the 1:100 dilution. The results of the 1:5 dilution in this study were highly variable, potentially due to blood clots. However, this dilution yielded comparable amounts in the first study, so the 56°C for 1 hour incubation period was incorporated in the protocol. The comparison of the spin baskets showed comparable DNA yields at the 1:100 dilution but significantly less DNA at the 1:5 dilution. However, in this experiment, sample remained in some of the spin baskets after centrifuging. Therefore, this was tested again in combination with the altered incubation period. Comparable DNA yields were obtained at both dilutions of whole blood. Therefore, these were both incorporated into the protocol. These studies should likely be repeated, and the speed of centrifugation could be increased to ensure sample flows through the spin baskets.

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