Comparison of Manual Stool DNA Isolation to Automated Stool DNA Isolation Using Norgen's Magnetic Bead Stool DNA Isolation Kit

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Application Note 102

INTRODUCTION

Research over the past few decades has revealed that the intestinal microbiome has a large influence on a variety of diseases ranging from obesity, type 2 diabetes, cardio-metabolic diseases,[1] mental health and neurodegenerative diseases,[2,3] to recent discoveries of gut microbiota modulating responses to cancer immunotherapy.[4] Microbiome research has now expanded to almost all areas of biomedical research. Large multi-omics studies are being performed to get a better understanding of the mechanisms involved. To support research in this area, automated workflows are essential for providing robust datasets with large sample sizes. Norgen's Stool DNA Isolation 96-Well Kit (Magnetic Bead System) (Cat. 63100) has been optimized for use by the Hamilton Vantage Liquid Handler.

The purpose of this study was to compare DNA isolated using a new automated protocol on the Hamilton Vantage using Norgen's Stool DNA Isolation 96-Well Kit (Magnetic Bead system) (Cat. 63100) to Norgen's manual method using magnetic beads and a competitor's manual method that uses a spin column. The results indicate that the automated system yielded 6X more DNA (~ 200 ng/μL) than the manual methods, and that the DNA derived from the automated method was of high purity, as indicated by the lack of PCR inhibition up to 8 μL of template addition.

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MATERIALS & METHODS

Sample Collection and Preservation

A stool sample was collected and stored in Norgen's Stool Nucleic Acid Collection and Preservation Tubes (Cat. 45660) and homogenized through vortexing. The preserved stool was aliquoted into 48, 200 μL samples; 16 samples for 3 different methods.

DNA Extraction

MoBio - The MoBio PowerFecal Kit was used following the manufacturer's instructions.

Manual - Norgen - The Norgen Stool DNA Isolation Kit (Magnetic Bead System) (Cat. 55700) was used and the kit protocol was followed.

Automated - Norgen - Norgen created an automated method on the Hamilton Vantage that uses the Norgen Stool DNA Isolation 96-Well Kit (Magnetic Bead System) (Cat. 63100). The automated stool DNA isolation method involves a pre-processing lysis step using bead beating. This is followed by a streamlined liquid handling method that binds the DNA to magnetic beads. It then performs an optimized washing protocol, followed by the elution of DNA from the beads.

DNA Quantification

Nucleic acid concentrations and quality were determined using 2 μL of eluate on the nanodrop. Furthermore, 10 μL of the DNA eluate was run on a 1.2% agarose gel at 170 V for 25 minutes.

16S qPCR

qPCR was performed in 20 μL reactions with Norgen TaqMan 2X PCR Master Mix (Cat. 28340) and 2 μL of 16S primer probe mix. Different volumes of eluate were added ranging from 2-8 μL. qPCR was performed on a BioRad CFX96 real-time system.

NGS Sequencing

Sequencing libraries were prepared using Norgen's 16S V3-V4 Library Preparation Kit for Illumina (Cat. 70400), and sequenced using MiSeq Reagent Nano Kit v3 (600 cycles).

RESULTS

DNA Yield

The automated Norgen method using the Hamilton Vantage resulted in much higher yield compared to the manual methods; 260 ng/μL compared to 33 ng/μL (Figure 1). Moreover, the 260/280 and 260/230 suggests good purity. The agarose gel also confirms high yield (Figure 2).
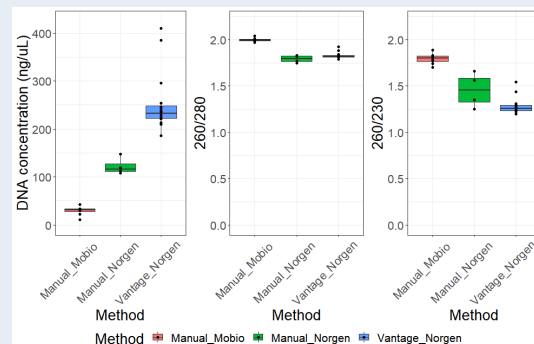

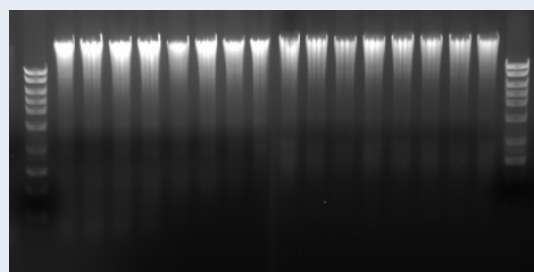**Figure 1.** DNA yields and purity as measured by nanodrop.

Figure 2. High-quality DNA isolated using the automated Norgen Magnetic Beads System and the Hamilton Vantage. 10 μL of DNA was run on a 1.2% agarose gel at 170 V for 25 minutes.

qPCR Results

Firstly, the qPCR results support the nanodrop quantifications as the automated Norgen method has the lowest Cqs, indicating the highest DNA concentration (Figure 3). Moreover, qPCRs of increasing template concentrations confirm high-quality eluate with negligible PCR inhibitors in the samples. For each doubling of the input template (from 2,4,8 µL) there is an approximate decrease of 1 Cq, which corresponds to a theoretical doubling of the template per cycle (Figure 4.)

Sequencing results

In total, 16 different genera were detected in the manual and automated samples. The community composition of the different replicates is very similar for both the Norgen-manual and Norgen-automated samples (Figure 5). There is also strong agreement between the manual and the automated samples, as permutational ANOVA analysis did not show any significant difference between the manual and automated samples. Only the Lachnospiraceae_unclassified genus is under-represented in the automated samples as compared to the manual samples (F: -10.5, pval < 0.05). However, an alternate genus in the Lachnospiracea class was identified in the automated samples, and not in the manual samples.

CONCLUSION

All of the data together shows that the automated method using Norgen's Stool DNA Isolation 96-Well Kit (Magnetic Bead System) (Cat. 63100) on the Hamilton Vantage offers a reliable, rapid, high throughput method that yields a high quantity of high-quality DNA from stool samples.

The extracted DNA is of high-quality and free of inhibitors as demonstrated by nanodrop as well as qPCR with increasing eluate concentrations. Moreover, the DNA extracted using the automated method yields an accurate picture of the microbial community composition as compared to DNA extracted using manual methods.

Therefore, automating DNA extraction will provide a reliable method to scale-up DNA extractions and provide the sample numbers necessary for statistically significant research questions.
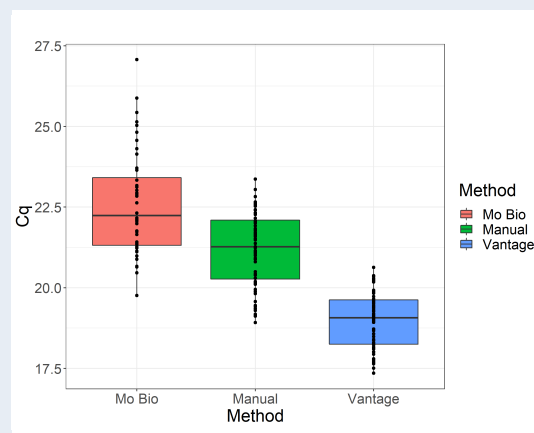

Figure 3. The DNA isolated using the Norgen automated kit had significantly lower Cqs supporting the higher DNA yield. Data for 2µL of eluate shown in this graph.
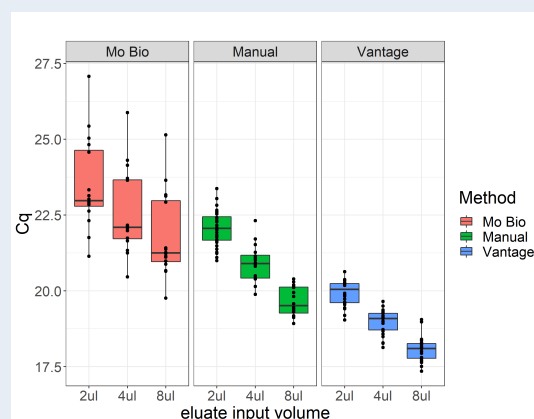

Figure 4. Cqs decrease with increasing template volume indicating negligible PCR inhibition.
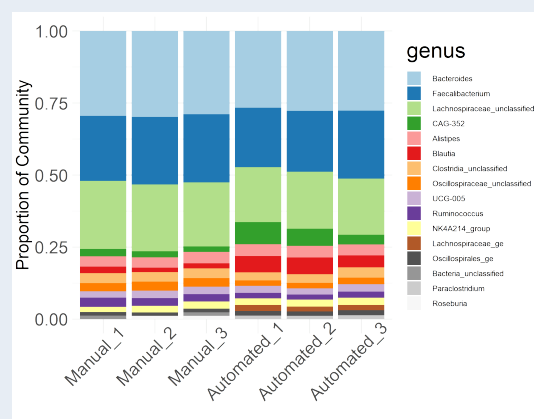

Figure 5. Community composition as determined by Illumina sequencing with V3-V4 primers. Manual_1:3 are replicate extractions from the Norgen-manual method, and Automated_1:3 are replicate extractions using the Norgen-automated method. There is good replicability between sample types and good agreement between the community as revealed through manual and automated DNA isolation.

REFERENCES

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Cat No.	Related Products	Size
55700	Stool DNA Isolation Kit (Magnetic Bead System)	50 Preps
45660	Stool Nucleic Acid Collection and Preservation Tubes	50 Tubes
63100	Stool DNA Isolation Kit 96-Well Kit (Magnetic Bead System)	2 x 96-Well Plates
28340	TaqMan 2x PCR Master Mix	100 rxns
70400	16S V3-V4 Library Preparation Kit for Illumina	24 Preps

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