Automation of Saliva DNA Isolation on the Hamilton Vantage Using Norgen's Magnetic Bead Saliva Isolation Kit

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Application Note 101

Keywords

+ Automation
+ DNA
+ Isolation
+ Saliva
+ Mag Bead
+ Comparison
+ 16S qPCR
+ NGS

INTRODUCTION

Recent research has revealed that the salivary microbiome is individualized, influenced by diet and lifestyle and reflects health status.[1,2] Studies have shown links between microbial composition and oral disease, diabetes, inflammatory diseases, cardiovascular disease and mental health.[3,4,5,6,7] The composition of the salivary microbiome might thereby mirror oral and general health status. Large multi-omics studies are being performed to better understand the mechanisms involved. To support research in this area, automated workflows are essential for providing robust datasets with large sample sizes. Norgen's Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) (Cat. RU62900) 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 Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) (Cat. RU62900) to Norgen's manual method using the Saliva DNA Isolation Kit (Magnetic Bead System) (Cat. RU55400). The results indicated that the automated system yielded more DNA than the manual method and that the DNA derived from the automated method was high purity, as indicated by the lack of PCR inhibition up to 8 µL of template addition. Furthermore, the eluted DNA is also suitable for metagenomic sequencing, and the automated samples yielded a similar community composition to the manually extracted samples.

MATERIALS & METHODS

Sample Collection and Preservation

Saliva samples were collected from 16 individuals and stored in Norgen's Saliva DNA Collection and Preservation Devices (Cat. RU49000). The preserved saliva was pooled and aliquoted into 32 samples: 16 samples for each of the two methods.

DNA Extraction

Manual - Norgen: The Norgen Saliva DNA Isolation Kit (Magnetic Bead System) (Cat. RU55400) was used, and the kit protocol was followed.

Automated - Norgen: An automated method was developed on the Hamilton Vantage using Norgen's Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) (Cat. RU62900). The automated saliva DNA isolation method involves a pre-processing incubation step to help liquefy sputum to prevent clogging. This is followed by a streamlined liquid handling method that binds DNA to the magnetic beads, performs an optimized washing protocol and then elutes the 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 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 a slightly lower yield compared to the manual methods, 104 ng/µL as compared to 118 ng/µL. However, the 260/280 and 260/230 suggests that the automated method yields an eluate of better quality (Figure 1). The agarose gel also confirms a high yield (Figure 2).

qPCR Results

Moreover, qPCRs of increasing template concentrations confirm high-quality

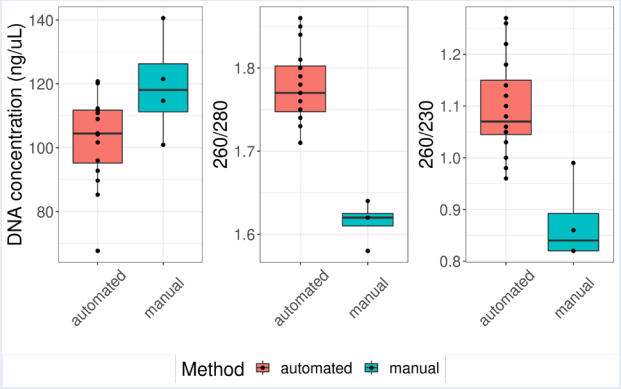

Figure 1. DNA yields and purity as measured by nanodrop.

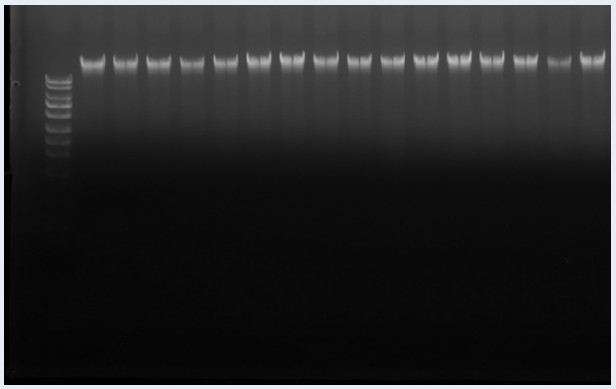

Figure 2. High-quality DNA was isolated using the automated method with Norgen's Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) on the Hamilton Vantage.

eluate with negligible PCR inhibitors in the samples. For each doubling volume 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 3).

Sequencing Results

In total, 12 different genera were detected in the manual and automated samples. The community composition of the different replicates is very similar within the Norgen-manual and Norgen-automated sample types (Figure 4). There is also a 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. Two genera are only found in the automated samples (Atopobiaceae and Actinomyces); however, these were found at 1% in the automated samples, which could be due to differences in sequencing depth. Only the Prevotellaceae_unclassified genus is under-represented in the automated samples compared to the manual samples (F: -8, pval_adj =0.02).

CONCLUSION

All of the data together show that the automated method using Norgen's Saliva DNA Isolation 96-Well Kit (Magnetic Bead System) (Cat. RU62900) on the Hamilton Vantage offers a reliable, rapid, high throughput method that yields a high quantity of high-quality DNA from saliva samples.

The extracted DNA is high-quality and free of inhibitors, as demonstrated by nanodrop and qPCR with increasing eluate concentrations. Moreover, the DNA extracted using the automated method yields an accurate picture of the microbial community composition compared to DNA extracted using manual methods. Therefore, automating DNA extraction from saliva will provide a reliable method to scale up DNA extractions and provide the sample numbers necessary for statistically significant research questions.

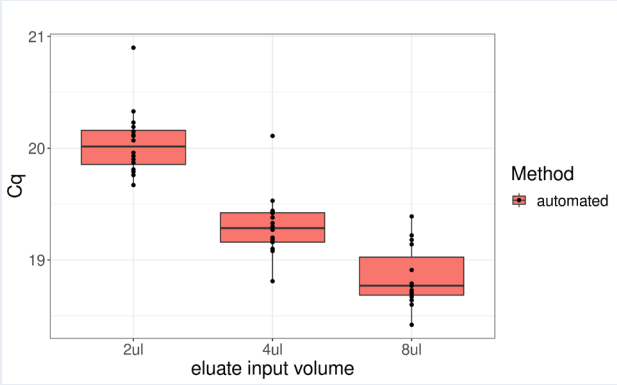

Figure 3: Cq's decrease with increasing template volume, indicating negligible PCR inhibition.

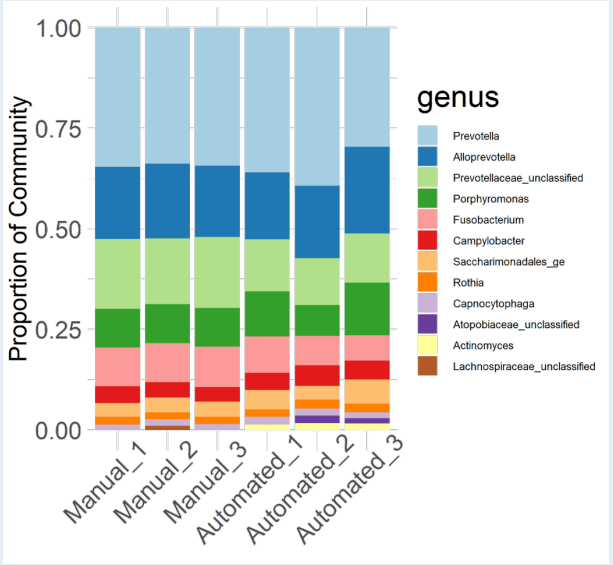

Figure 4: The bar graphs show the relative abundance of genera detected in the saliva samples. The bacterial community composition is similar between the manual and automated samples and consistent between replicates. Sequencing libraries were prepared using Norgen's 16S V3-V4 Library Preparation Kit for Illumina and sequenced using MiSeq Reagent Nano Kit v3 (600 cycles).

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Cat No.	Related Products	Size
RU55400	Saliva DNA Isolation Kit (Magnetic Bead System)	50 Preps
RU62900	Saliva DNA Isolation Kit (Magnetic Bead System) 96-Well Format	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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