Linearity of DNA Isolated from Increasing Volumes of Preserved Saliva Using Norgen's Saliva DNA Isolation Kit

APPLICATION NOTE #54

M. EL-MOGY, PH.D[1] , M. SIMKIN[1] , Y. HAJ-AHMAD, PH.D[1,2]
[1]NORGEN BIOTEK CORPORATION, THOROLD, ONTARIO, CANADA
[2]CENTRE FOR BIOTECHNOLOGY. BROCK UNIVERSITY, ST. CATHARINES, ONTARIO, CANADA

INTRODUCTION

Saliva is a useful bodily fluid for diagnostic and research purposes. Collection is non-invasive and practical, as DNA isolated from saliva can be used for the screening and detection of biomarkers of cancer and autoimmune disorders, as well as for genotyping and more[1,2] .

Norgen Biotek Corp. has developed a simple method for the collection, preservation, and storage of DNA from saliva using Individual Saliva DNA Collection and Preservation Devices (Cat# 35710). Donors simply collect their saliva directly into the Collection Tube and add Norgen's Saliva DNA Preservative. The preservative is an aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of saliva DNA from fresh specimens. This buffer stabilizes the DNA for long-term storage at ambient temperatures. Since the buffer prevents the growth of microorganisms and inactivates viruses, it also allows the samples to be handled and shipped safely. Congruently, Norgen has also developed a Saliva DNA Isolation Kit that is fast, reliable, and very customer-friendly, eluting saliva DNA of the highest quality and yield that can be used directly in sensitive downstream diagnostic assays such as PCR.

Investigators utilizing saliva in their research have unique needs, based on their downstream applications and sample volume. For some studies, less saliva may be processed in order to preserve precious samples. On the other hand, some studies require a high yield of DNA from their samples, and thus require higher volumes of sample to be processed. Therefore, investigators must ensure that their saliva DNA isolation method is flexible, i.e. a linear increase in saliva volume being processed leads to a linear increase in saliva DNA concentration and yield. A robust saliva DNA isolation kit eliminates sample processing biases, and increases data reproducibility.

The purpose of this study is to demonstrate the flexibility of Norgen's Saliva DNA Isolation Kit by processing increasing volumes of preserved saliva.

METHODS AND MATERIALS

Sample collection

Four milliliters of saliva was collected from three different participants. All samples were preserved in Norgen's saliva preservative, and pooled together.

Saliva DNA extraction

DNA was extracted from all saliva samples using Norgen's Saliva DNA Isolation Kit (Cat# 45400), as per the manufacturer's instruction. Briefly, saliva samples were incubated at 55°C for 1 hour prior to DNA isolation. After inverting each saliva sample, 50µL, 100µL, 250µL or 500µL of preserved saliva was transferred to a new microcentrifuge tube. Samples were then incubated at 55°C for 20 minutes with 20 µL of proteinase K, binding solution was added along with ethanol, and samples were bound, washed and eluted as per manufacturer's instruction.

Spectrophotometry

Saliva DNA quantity and quality was measured using the UltraSpec 2100 Pro (Fisher Scientific). Briefly, 50µL of each saliva DNA elution was diluted with 450µL of nuclease-free water, and OD measurements were taken using the cuvette-based spectrophotometry method.

Real-Time PCR

The purified DNA was then used as the template in a realtime TaqMan PCR reaction. Briefly, 9µL of isolated DNA was added to 20µL of real-time PCR reaction mixture containing 10µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR® Green dye, 5 mM GAPDH primer pair, and nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (BioRad).

RESULTS AND DISCUSSION

The use of saliva as a diagnostic medium is steadily increasing, as collection is easy and non-invasive. As a result, the need for a reliable, robust saliva DNA isolation method is increasingly becoming apparent. One method of determining the reliability of a saliva DNA isolation method is consistency, while another important method, demonstrated in this study, is scalability and linearity of DNA isolated from increasing volumes of saliva.

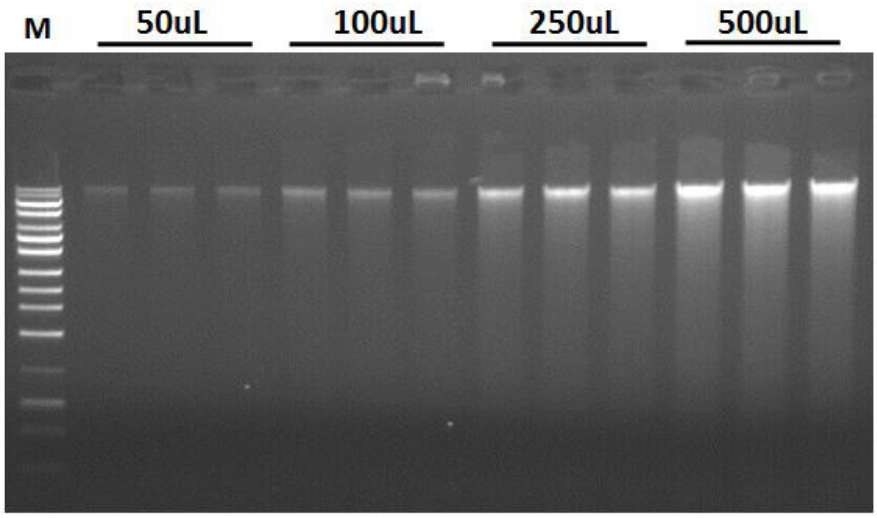

Figure 1. Resolution of DNA isolated from pooled saliva collected from 3 donors preserved using Norgen's Saliva Preservative, and isolated using Norgen's Saliva DNA Isolation Kit. Twenty microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel. M= Norgen's UltraRanger DNA Ladder.

In this study, saliva was collected from three different participants, preserved in Norgen's saliva preservative, and pooled together. DNA was then isolated from 50µL, 100µL, 250µL and 500µL aliquots of the preserved saliva, and run on a 1.0%, 1X TAE agarose gel (Figure 1). The increase in saliva DNA yield from increasing volumes of saliva being processed is clearly demonstrated in Figure 1, with the relationship between sample volume and DNA yield being evidently linear. DNA concentrations were then measured using the UltraSpec 2100 Pro (Fisher Scientific), with calculated DNA yields correlating with the gel photo in Figure 1 (Figure 2). Once again, the linear relationship between increasing volumes of saliva processed and DNA yields can be observed.

One concern with altering sample volumes would be a change in DNA quality. High quality saliva DNA is required in most sensitive downstream applications, and is thus extremely important when evaluating a saliva DNA isolation method. Two methods were used in this study to demonstrate DNA quality: the A260:A280 ratio, and the Ct values generated from a TaqMan qPCR reaction.

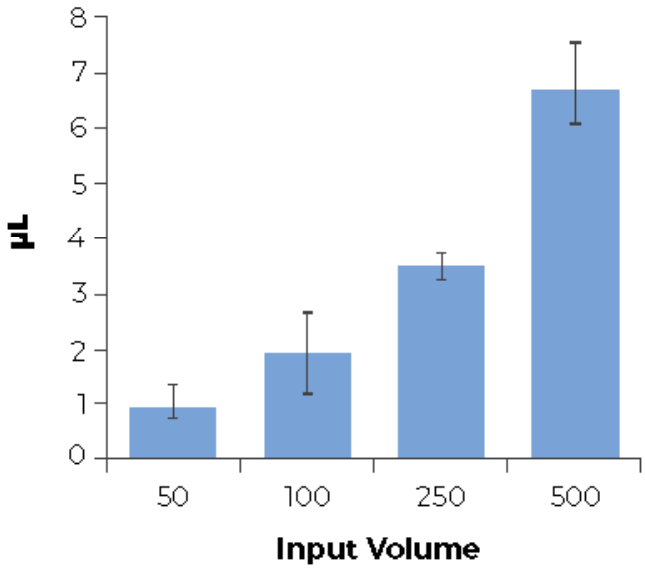

Figure 2. Linear increase in DNA yield corresponding to a linear increase in preserved saliva volume processed. Fifty microliters of each sample was diluted in 450µl of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

The A260:A280 ratio is a common measurement of nucleic acid quality. In this study, a cuvette-based spectrophotometry method was used to determine the A260:A280 of the saliva DNA samples (UltraSpec 2100 Pro; Fisher Scientific). The A260:A280 values were then graphed, and can be visualized in Figure 3. Propitiously, the A260:A280 values were not significantly affected by altering the starting volume of saliva, and with the average A260:A280 of all samples being ~1.7, this is indicative of minimal to no RNA contamination.

The final method of DNA quality determination was through the use of a TaqMan Real-Time PCR method. In order to assess sample inhibition, 9µL of sample was used in the reaction. The Ct values were then graphed, and depicted in Figure 4. As can be seen, the samples exhibited virtually no inhibition, and the decrease in Ct values was linear with the increase in the volume of saliva processed.

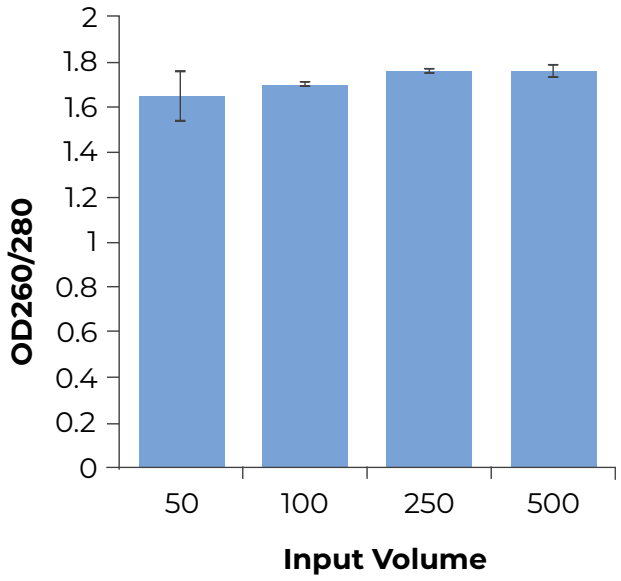

Figure 3. Virtually no change in A260:A280 when sample volume is adjusted. Fifty microliters of each sample was diluted in 450µL of nuclease-free water, and spectrophotometry measurements were taken using the UltraSpec 2100 Pro (Fisher Scientific).

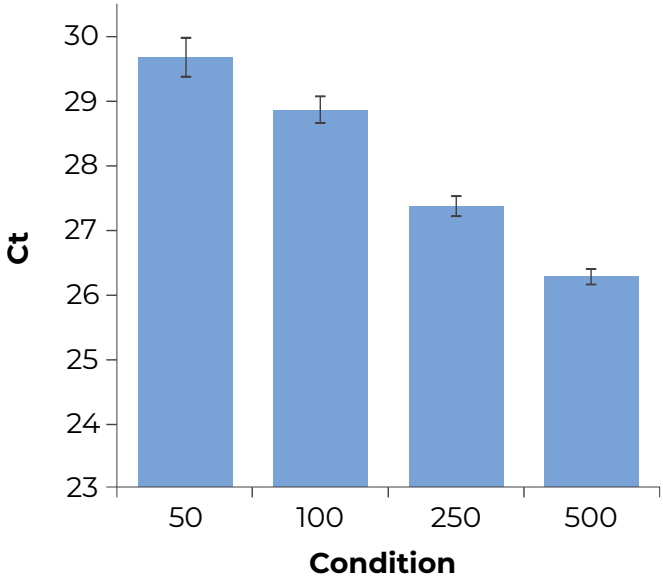

Figure 4. Linear decrease in Ct value corresponding to a linear increase in preserved saliva volume processed. Nine microliters of saliva DNA template was used in a 20µL TaqMan qPCR reaction involving GAPDH primers, using the iCycler Thermal Cycler (BioRad Laboratories).

CONCLUSIONS

From the data presented in this report, the following can be concluded:

1. Norgen's Saliva DNA Isolation Kit can be used to Isolate DNA from a Range of Sample Volumes. In this report, we demonstrate the ability to isolate DNA from 50-500µL of preserved saliva, with a linear increase in DNA corresponding to a linear increase in volume of saliva processed.
2. Norgen's Saliva DNA Isolation Kit is Consistent and Reliable. We have demonstrated through the use of multiple sample replicates that Norgen's kit consistently isolates high quality DNA across varying volumes of input saliva.
3. DNA Isolated from Norgen's Saliva DNA Isolation Kit is of the Highest Quality, with Minimal to No RNA Contamination. As shown in this report, when 50µL to 500µL of preserved saliva was used as the input, Norgen's kit elutes DNA with an A260:A280 of ~1.7, indicating high quality DNA with limited RNA contamination.
4. Norgen's Saliva DNA Isolation Kit Elutes DNA Ready for Sensitive Downstream Applications. When 9µL of sample was used in a sensitive TaqMan qPCR reaction, no inhibition was observed, and Ct values corresponded to the volume of saliva processed, in a linear fashion.

REFERENCES

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