DNA Isolation from Saliva Preserved with Norgen's Saliva DNA Collection and Preservation Device using a Competitor's DNA Blood Kit

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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] .

Competitor's DNA blood kit utilizes a spin-column format containing a silica-gel membrane to capture DNA from many sample types, including saliva. It is a common kit used by researchers to isolate genomic DNA from 200 µL of saliva. It has been used to isolate saliva DNA from buccal swabs to identify infections in canines[3], and to isolate saliva from various paper types for forensics[4]. It has also been used to isolate saliva DNA for HHV-8 and HIV detection[5].

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. The DNA subsequently isolated from the preserved samples is of a high quality and can be used directly in sensitive downstream diagnostic assays such as PCR.

The purpose of this study is to determine the compatibility of Norgen's Saliva DNA Collection and Preservation Device with a competitor's DNA blood kit.

MATERIALS AND METHODS

Sample collection

Four milliliters of saliva was collected from two different participants. Both samples were preserved in Norgen's saliva preservative.

Saliva DNA extraction

DNA was extracted from all saliva samples using either Norgen's Saliva DNA Isolation Kit (control; Cat# 45400) or the competitor's DNA blood kit, 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, 200 µL of preserved saliva was added to new microcentrifuge tubes. Samples being isolated using the Norgen Saliva DNA Isolation Kit were 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. For the competitor's DNA blood kit, samples were mixed with 20 µL of protease (supplied with the kit), 200 µL Buffer AL, and incubated for 10 minutes at 55°C. After the additional of ethanol, samples were bound washed and eluted as per manufacturer's protocol.

Real-Time PCR

The purified DNA was then used as the template in a realtime PCR (qPCR) reaction. Briefly, 2 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR® Green dye, 2.5 mM 5S 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 72°C for 45 seconds for extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

Saliva DNA was isolated from two different saliva samples, isolated in duplicate using the Norgen Saliva DNA Isolation Kit, and in triplicate using the competitor's DNA blood kit. The Norgen kit was used as a positive control, as the Norgen saliva preservative has been optimized for this kit. Fifteen microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel **(Figure 1).** It was found that the DNA isolated using the competitor's DNA blood kit was equivalent with regards to yield and purity to the Norgenisolated samples (which the preservative was optimized for). Next, to determine the quality of the DNA isolated using both systems, 2 µl of purified DNA were used in a 20 µl qPCR reaction using Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR Green® (Bio-Rad), using 5s rRNA primers **(Figure 2).** Based on the Ct values generated, the Norgen-isolated saliva and the competitor-isolated saliva were of comparable quality, with all samples amplifying at a Ct of ~20. Both kits were found to isolate consistent DNA quality and quantity, as both were found to have low standard deviations as well. This indicates that Norgen's Saliva DNA Preservative preserves DNA consistently, and is compatible with many competitor kits, including the competitor's DNA blood kit.

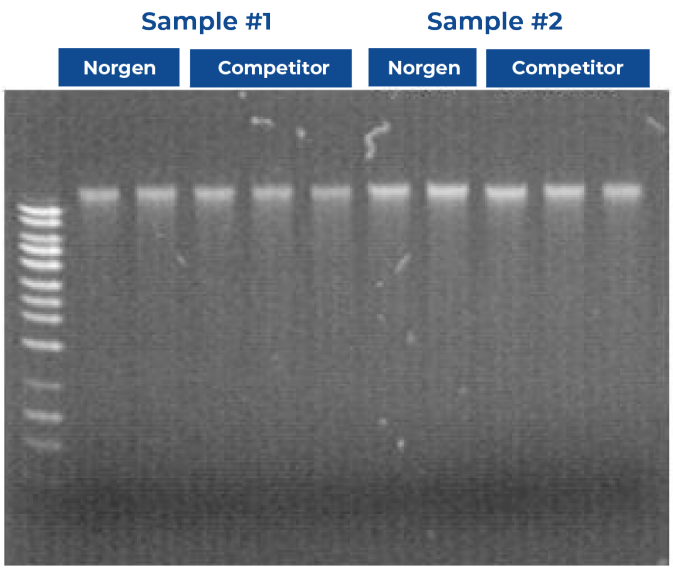

Figure 1. Saliva DNA yield from two donors, preserved using Norgen's Saliva Preservative, and isolated using both the Norgen Saliva DNA Isolation Kit and the competitor's DNA blood kit. Fifteen microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel.

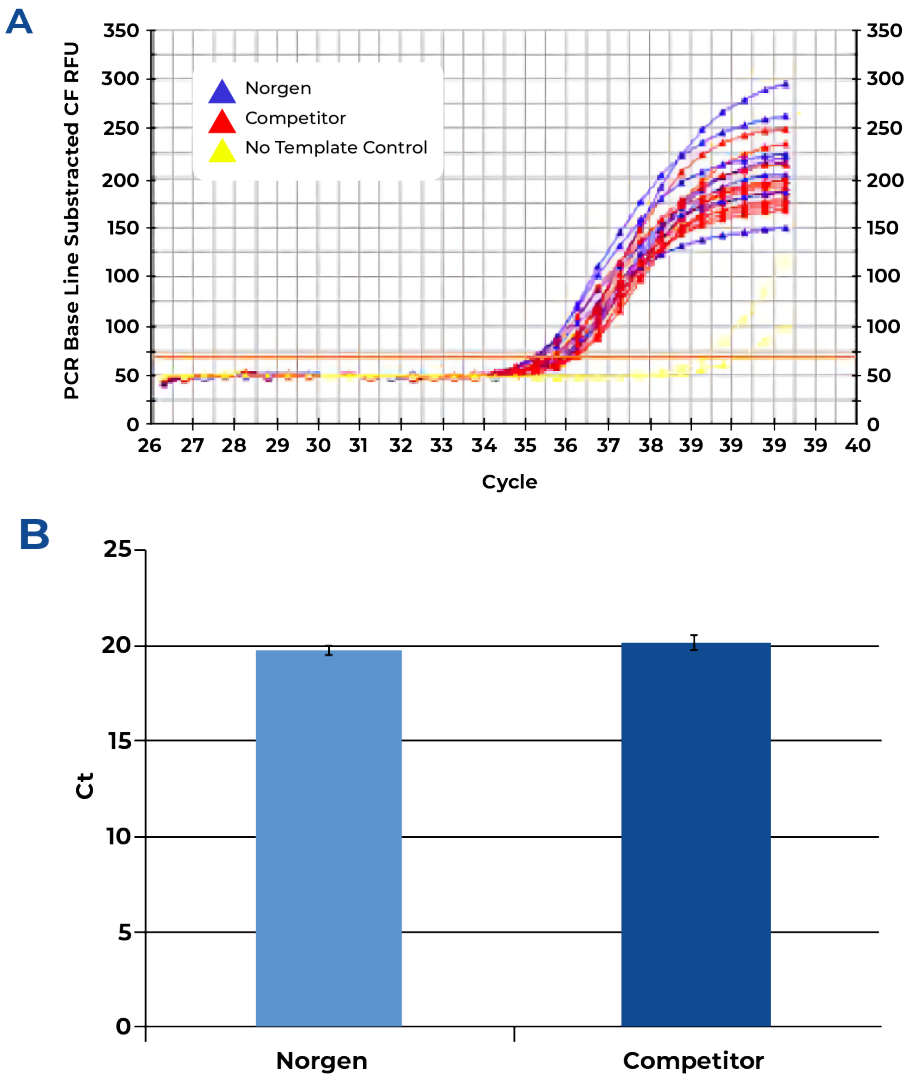

Figure 2. Real-time PCR of DNA preserved in Norgen's saliva preservative, isolated from both the Norgen Saliva DNA Isolation Kit and the competitor's DNA blood kit. Two microliters of purified DNA were used in a 20 µl qPCR reaction using Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR Green®, using 5s rRNA primers. A) The qPCR amplification plot; B) A bar graph representation of the average Ct observed for Norgen-and competitor-isolated saliva DNA.

CONCLUSIONS

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

1. **Norgen's Saliva DNA Preservative is Compatible with Many Commercially Available Saliva DNA Isolation Kits.** We have found Norgen's saliva DNA preservative to be compatible with most saliva DNA isolation systems. Here, we have shown Norgen's preservative to be compatible with the competitor's DNA blood kit, generating high quality DNA with comparable yields to the Norgen's Saliva DNA Isolation Kit (Cat# 45400).

2. **Norgen's Saliva DNA Preservative Optimally Performs across a Variety of Saliva Samples.** Norgen's preservative allowed for the isolation of high quality and yields of DNA from different samples, using two different saliva DNA isolation kits. This ability to isolate high yields from a variety of samples has been found using numerous isolation methods, including the competitor's DNA blood kit.

3. **Norgen-Preserved Saliva DNA is Consistent.** When multiple replicates of Norgen-preserved saliva was used in two different DNA isolation systems, standard deviations generated from qPCR Ct values were small, indicating consistently high quality DNA.

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