Comparison of Norgen's Saliva DNA Collection, Preservation and Isolation Kit to a Leading Competitor's Kit

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INTRODUCTION

In recent years attention has been turning to the use of non-invasive samples for genetic and diagnostic analysis, including the use of saliva. Norgen Biotek Corp. has developed a simple method for the collection, preservation, storage and purification of DNA from saliva using Individual Saliva DNA Collection and Preservation Devices. 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 temperature. 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 real-time PCR. In this application note Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700**)** is compared to the leading competitor's kit in terms of yield, purity, and compatibility with downstream applications.

METHODS AND MATERIALS

DNA Isolation

Five different saliva samples were collected and 2 mL of saliva was preserved using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700) and 2 mL was preserved using a leading competitor's preservation buffer for 1 day at ambient temperature. After 1 day saliva DNA was isolated according to each of the supplied protocols.

Determination of DNA Yield

DNA yield and purity were determined by NanoVue Plus™ (GE Healthcare) according to the manufacture manual.

Gel electrophoresis

For visual analysis of DNA size and integrity, 10 µL of DNA from each of the final DNA elutions was loaded on a 1% agarose TAE gel and run for 25 minutes at 150 V. Gel photo was taken by AlphaImagerTM IS-2200 (Alpha Innotech).

Real-Time PCR

One hundred nanograms of each of the isolated DNA samples were used as the template of a real-time PCR reaction using 2X SYBR Green/Fluorescein qPCR Master Mix (Norgen Biotek). GAPDH specific primers (forward: 5'accacagtccatgccatcac3'; reverse: 5'tccaccaccctg ttgctgta3', amplicon size of 452bp) were used in the reaction amplified on the iCycler iQ real-time system (Bio-Rad).

RESULTS AND DISCUSSION

Norgen's Saliva DNA Collection, Preservation and Isolation Kit was compared to the leading competitor's kit by collecting 5 different saliva samples and adding 2 mL of each sample to 2 mL of each kit's preservation buffer. After 1 day of storage at room temperature total saliva DNA was isolated from each 4 mL of saliva/preservative mix using each of the provided protocols and reagents. The DNA yield was determined using a NanoVue Plus™ (GE Healthcare) according to the manufacture manual. By observing **Figure 1** below it can be seen that Norgen's kit isolated a higher yield of DNA. The average DNA yield was 44.15 µg and 43.75 µg from Norgen's Saliva DNA Collection, Preservation and Isolation kit and the competitor's kit, respectively.

In order to compare the integrity, yield and size of the DNA isolated using the 2 different kits aliquots of the purified DNA were also run on 1% agarose TAE gels for visual analysis. From observing the gel in **Figure 2**, it can be seen that indeed Norgen's kit did result in a higher yield of DNA that was of a high integrity.

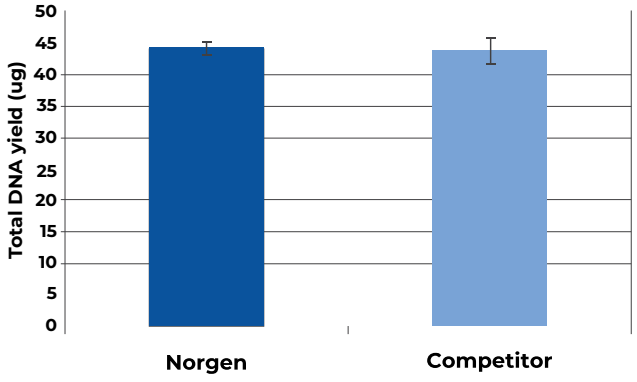

Figure 1. Graph comparing the total DNA yield from Norgen's kit and a leading competitor's kit as measured by spectrophotometer. DNA was isolated from 4 mL of saliva/preservation mixture as per the provided protocols and reagents.

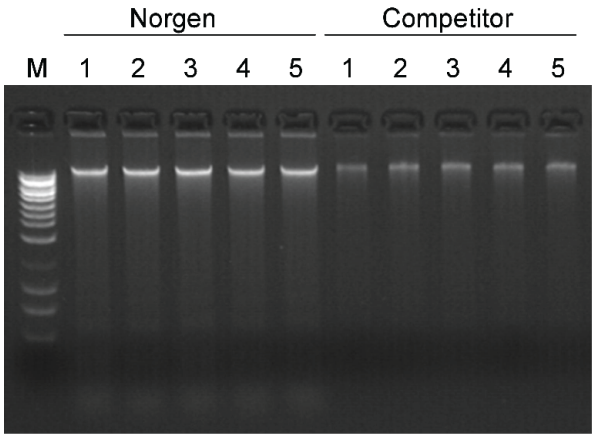

Figure 2. Agarose gel comparing the yield, size and integrity of saliva DNA isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kit and a competitor's kit. Norgen's kit resulted in higher yields of DNA from the same volume of saliva.

The 2 kits were then compared in terms of DNA purity. The purity of DNA is a critical factor which can affect downstream applications such as SNP analysis, digestion and PCR analysis. The OD at 260/280 is usually a key measurement for the DNA purity. **Figure 3** indicates that DNA samples isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kit showed higher average OD 260/280 values (1.67) than the competitor's kit (1.16), suggesting that Norgen's Saliva DNA Collection, Preservation and Isolation Kit provides a higher purity of DNA than the leading competitor.

Next, the purified DNA samples were compared using a common downstream application for saliva DNA, real-time PCR. The samples must be of a high quality in order to obtain successful real-time PCR results. Here GAPDH specific primers were used in the amplification of the saliva DNA samples on the iCycler iQ real-time system (Bio-Rad). As it can be seen in **Figure 4**, both Norgen-isolated and competitor-isolated DNA was successfully amplified in the real-time PCR reaction, indicating that both are suitable for use in sensitive downstream applications.

CONCLUSIONS

In this limited evaluation, Norgen's Saliva DNA Collection, Preservation and Isolation Kit was compared to the leading competitor's kit in terms of yield, purity, and compatibility with downstream applications. Norgen's kit resulted in a slightly higher average yield of DNA, as the average DNA yield from Norgen's kit was 44.2 µg and the average yield from the competitor's kit was 43.8 µ g. This was further verified by agarose gel analysis. Norgen's kit showed higher average 260/280 OD values (1.67) than the competitor's kit (1.16), suggesting that Norgen's kit provides a higher purity of DNA than the leading competitor. Lastly, both Norgen-isolated and competitorisolated DNA was successfully amplified in a real-time PCR reaction amplifying the GAPDH gene, indicating that both are suitable for use in sensitive downstream applications.

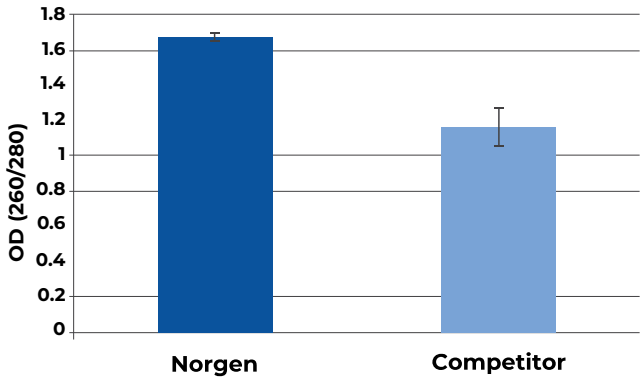

Figure 3. Graph comparing DNA purity from Norgen's kit and a leading competitor's kit by measuring OD 260/280. Norgen's Saliva DNA Collection, Preservation and Isolation Kit produces a higher quality of DNA than the leading competitor kit.

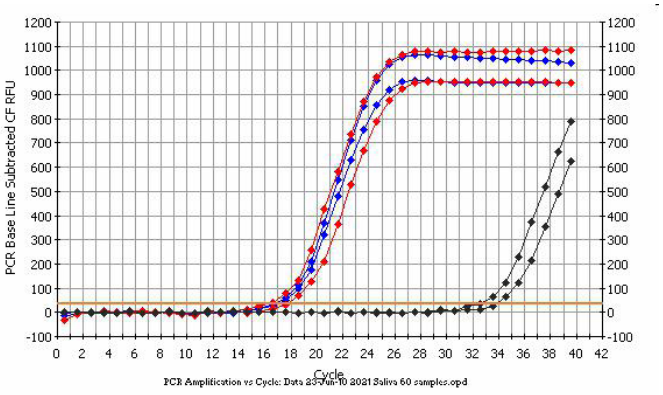

Figure 4. Real-time PCR analysis with GAPDH primers. Both the DNA samples from Norgen's Saliva DNA Collection, Preservation and Isolation Kit (blue) and the competitor's kit (red) were amplified, indicating their suitability for use in sensitive downstream applications. The black line represents the no-template control (NTC).

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