A Comparative Study Between Two Saliva DNA Preservation Systems, and Two Column-Based Saliva DNA Purification Methods

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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].

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# 49000). 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. Competitor O's DNA saliva collection device works similarly to Norgen's saliva collection system, however the preservatives contained within the two collection devices are different. As these two preservation systems vary in composition, choosing a specific preservation system can impact the quantity and quality of the saliva DNA isolated.

Currently, there are two main categories of saliva DNA isolation methods: a spin column format and alcohol precipitation. In this study, we looked at two popular spin column methods for saliva DNA isolation, as well as the alcohol precipitation method. This report will focus on the spin column methods. The two spin column methods used include: Norgen's Saliva DNA Isolation Kit (Cat# 45400) and Competitor Q's DNA Blood Mini Kit (Cat# 51104).
The purpose of this study is to assess the differences between Norgen's and Competitor O's saliva DNA preservation systems with regards to DNA quantity and quality when used on the two aforementioned purification systems.

MATERIALS AND METHODS

Sample Collection and Preservation

Two milliliters of saliva was collected from six different participants. All samples were preserved in 2 ml of either Norgen's saliva preservative or Competitor O's saliva preservative.

Saliva DNA Purification

Saliva DNA was purified using two different methods, with all methods being conducted by two different experimenters to assess experimenter variability and data reproducibility. DNA was extracted from all saliva samples using either Norgen's Saliva DNA Isolation Kit or the Competitor Q's DNA Blood Mini Kit, as per the manufacturer's instructions. Briefly, saliva samples were incubated at 55°C for 1 hour, prior to DNA isolation. After inverting each saliva sample, 400 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 Q's DNA Blood Mini Kit, samples were mixed with 20 µL of protease (supplied with the kit), 400 µL Buffer AL, and incubated for 10 minutes at 55°C. After the addition of ethanol, samples were bound, washed and eluted as per manufacturer's protocol.

Agarose Gel Electrophoresis and Quantification

Ten percent of each elution was loaded on a 1X TAE 1.2% agarose gel. This equates to 20 µl of spin-column elutions, and 10 µl of alcohol precipitation elutions. Two microliters of all elutions were also measured using the NanoVue Plus™ nanospectrophotometer to assess DNA concentration, A260:A280 and A260:A230 ratios.

Real-Time PCR

The purified DNA was then used as the template in a real-time 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 GAPDH

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Figure 5. Average Ct values generated from six purified saliva DNA samples, using Norgen's preservative compared to Competitor O's preservative, isolated using Competitor Q's isolation kit compared to Norgen's isolation kit, from two experimenters.

CONCLUSIONS

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

1. **No significant differences were found between Norgen's saliva DNA preservative compared to Competitor O's preservative**. Both preservatives were found to generate comparable DNA yield and quality from all saliva samples, using two different isolation methods.
2. **No significant differences found in DNA yields** isolated using Norgen's vs. Competitor Q's isolation kits.
3. **No significant differences found in A260:A280 ratios or Ct values** generated from Norgen's vs. Competitor Q's isolation kits.
4. **Significant differences were found in A260:A230 ratios** generated between Norgen's and Competitor Q's kits. Norgen was found to have statistically significantly higher average A260:A230 ratios, compared to Competitor Q's kit. However, as this was the only significant finding, it can be concluded that both kits are nearly identical in their generation of the highest quality and quantity of DNA from saliva.

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