The Use of Saliva Swabs in Norgen's Saliva DNA Collection, Preservation and Isolation 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]. One issue with using saliva as a diagnostic fluid is that some patients i.e. farm animals, infants, some elderly or mentally handicapped individuals, etc. are unable to collect saliva at all. For studies involving these individuals, saliva swabs have been found to be quite useful. Saliva swabs have been used to isolate DNA from infants into childhood for cohort studies[3], to compare saliva swab DNA from infants to DNA isolated from umbilical cords[4], and from dogs for forensic purposes[5].

Norgen Biotek Corp. has developed a simple method for the collection, preservation, and storage of DNA from saliva swabs using Individual Saliva DNA Collection and Preservation Devices (Cat# 35710) and individually wrapped saliva swabs (Cat# 49050). Researchers (or the donors) simply peel back the sleeve containing the swab, and place it in the donor's cheek pouch, rotate and move the swab head for 30 seconds to collect as much saliva as possible. This is repeated with a second swab, with both being broken at the pre-marked line, and placed directly into the collection tube. Norgen's Saliva DNA Preservative is then added to rapidly lyse cells and subsequently preserve the saliva DNA. The purpose of this study is to demonstrate the use of preserved saliva swabs for the isolation of both high quality and yield of DNA using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700).

MATERIALS AND METHODS

Sample Collection and Saliva DNA Extraction

Two different donors were used in this study to determine the robustness of the protocol. Two saliva swabs were collected from each donor. Donor A was a 23 year old female, and donor B was a 34 year old male. Swabs were preserved in 2mL of Norgen's saliva preservative. DNA was extracted from all preserved saliva swabs using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700) as per the manufacturer's instruction. Briefly, saliva samples were vortexed and incubated at 55°C for 1 hour, prior to DNA isolation. Samples were then incubated at 55°C for 30 minutes with 50 µL of proteinase K. Swabs were then removed, after pressing them against the tube wall to remove all liquid. Six hundred microliters of preserved saliva was then isolated in triplicate. Equal volumes of isopropanol was added to each sample, and samples were inverted 10 times prior to a 5 minute centrifugation at 14 000 x g. Supernatants were then discarded, and DNA pellets were washed with 70% ethanol. After 5 minutes of drying, saliva DNA was eluted in 100µl of Tris pH 11, and incubated at 55°C for 15 minutes to facilitate DNA hydration.

Agarose Gel Electrophoresis and Spectrophotometry

Ten microliters of purified saliva DNA was run on a 1X TAE 1.0% agarose gel, pre-stained with ethidium bromide, and viewed using the AlphaImager 2200 (AlphaInnotech). Samples were then quantified using the Ultraspec 2100 Pro spectrophotometer (Fisher Scientific). A two-tailed student t-test was used to determine statistical significance between DNA yields, A260/A280 and A260:A230 ratios, and Ct values.

Real-Time PCR

The purified DNA was then used as the template in a real-time PCR (qPCR) reaction. Briefly, 2µL 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, 250 nM 18S primer pair, and nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes, 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 45 seconds. The reaction was run using the iCycler iQ Real-time System (Bio-Rad).

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RESULTS AND DISCUSSION

Saliva DNA was isolated from saliva swab samples, from two different donors, in triplicate using Norgen's Saliva DNA Collection, Preservation and Isolation Kit. Fifteen microliters of 100µL elutions were run on 1X TAE 1.0% agarose gel **(Figure 1)**. It was found that one donor had a higher amount of DNA in their saliva than the other donor. This is quite common, as individuals will have varying amounts of DNA in their saliva for a number of reasons including: different environmental exposures, gender, method of saliva collection, and age.

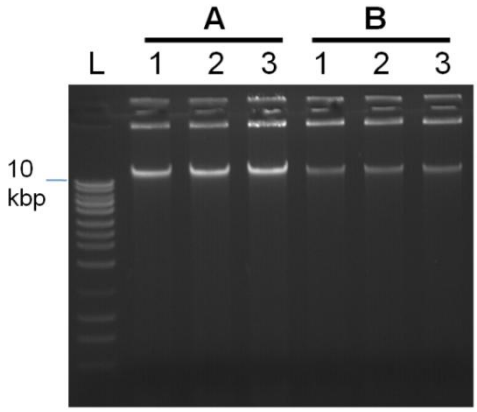

Figure 1. Purified saliva DNA isolated from swabs collected by two donors (A and B), run on a 1X TAE 1.0% agarose gel. High quality saliva DNA was isolated from both samples, with donor A showing a higher yield of DNA than donor B.

To determine the quantity of saliva swab DNA isolated, 5µl of DNA was diluted in 495µl nuclease-free water, and run through the Ultraspec 2100 Pro spectrophotometer (Fisher Scientific). Correlating with the gel, it was found that donor A had a significantly higher amount of saliva DNA than donor B **(Figure 2; p-value= 0.015)**.

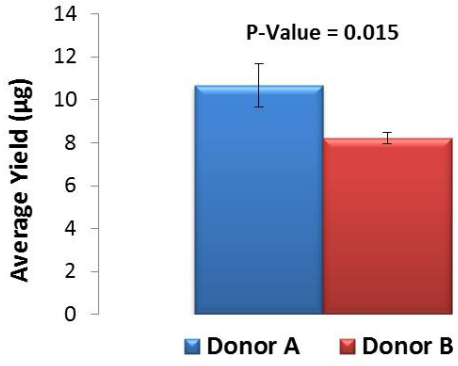

Figure 2. The average yields generated from saliva swab samples collected from two donors, analyzed using the Ultraspec 2100 Pro spectrophotometer (Fisher Scientific).

The A260:A280 and A260:A230 ratios, generated from spectrophotometry, were used to assess sample quality **(Figure 3)**. It was found that while donor B had a significantly lower amount of saliva DNA isolated compared to donor A, the quality of sample B was found to be significantly higher than sample A (A260:A280 p-value= 0.053; A260:A230 p-value= 0.001).

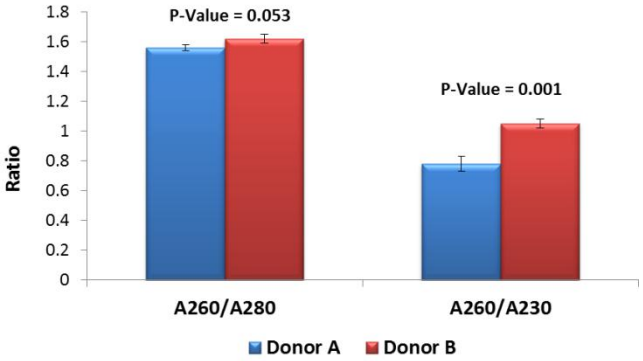

Figure 3. The A260:A280 and A260:A230 of purified saliva DNA isolated from swabs collected from two donors (A and B), analyzed using the Ultraspec 2100 Pro spectrophotometer (Fisher Scientific).

Finally, 2 µl of purified DNA was used in a 20 µl qPCR reaction flanking 18s rRNA primers **(Figure 4)**. Figure 4A shows the qPCR graph, while Figure 4B is a graphical representation of the average Ct values generated for the 3 sample replicates for both donors. The average Ct for donor A was found to be significantly lower than donor B (p-value= 0.011), correlating with the significant differences found in yield **(Figure 2)**. The lower quality of DNA isolated from donor A did not seem to affect Ct values.

The differences found in the average yield, quality, and Ct value between the two individual samples were consistent across the three sample replicates. All differences were found to be significant, facilitated by the small standard deviations that were generated. This indicates that DNA isolated from saliva swabs using Norgen's collection, preservation and isolation kit is consistent, and reliable, which is extremely important for diagnostic and forensic research.

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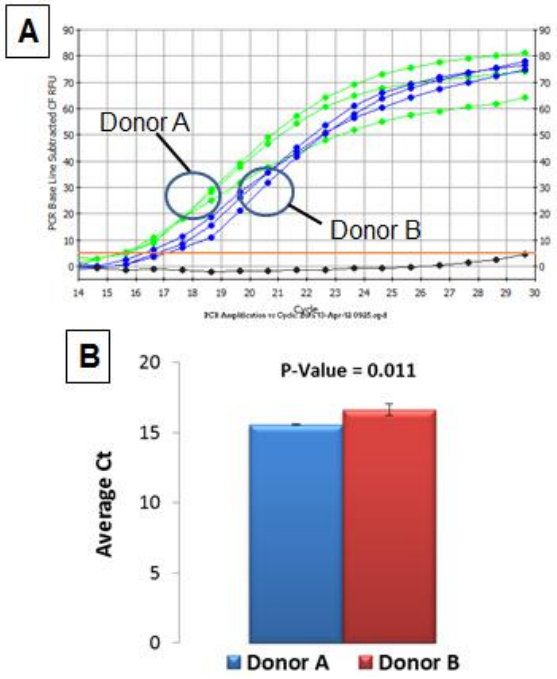

Figure 4. Real-time PCR of saliva DNA isolated from saliva swab samples collected from two donors, using the iCycler iQ Real-time System (Bio-Rad). A) The qPCR amplification graph. Donor A= green dots, Donor B= blue dots, NTC= black dots); B) The average Ct generated from both saliva swab samples, across 3 replicates.

CONCLUSIONS

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

1. **Convenience.** Saliva swabs are an excellent method for isolating high quality saliva DNA from individuals who cannot easily collect saliva.
2. **High quality DNA.** Norgen's Saliva DNA Collection, Preservation and Isolation Kit allows for rapid DNA extraction from saliva swab samples, producing high quality DNA ready for any downstream application.
3. **Consistency.** Norgen's Saliva DNA Collection, Preservation and Isolation Kit is very consistent and reliable, allowing for more precise DNA purification from a variety of saliva swab samples. This is also important for statistical analyses to be made between samples or groups.

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