DNA Isolation from Saliva Preserved using Norgen's Saliva DNA Collection and Preservation Device using Beckman Coulter Agencourt DNAdvance Kit

APPLICATION NOTE #44

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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 more1,2 . The Beckman Coulter Agencourt DNAdvance Kit is a popular kit for the purification of DNA from saliva for genotyping, as researchers have recently used it for genotyping the oxytocin receptor gene in mistreated children3 , and the serotonin receptor in posttraumatic stress patients4 .

The Agencourt DNAdvance Kit (Beckman Coulter, Danvers, MA) is a paramagnetic bead system, built on Solid Phase Reversible Immobilization (SPRI). It is useful for highthroughput studies, it is compatible with automated work stations, and it does not require centrifugation or vacuum manifold. In this study, we compared DNA isolation from Norgen-preserved saliva and Oragene-preserved saliva using the Agencourt DNAdvance Kit to determine the compatibility of both preservatives on this unique system.

MATERIALS AND METHODS

Sample collection

Saliva samples were collected from three different individuals. Four milliliters of saliva was collected from each participant. Half of each sample was preserved in Norgen's saliva preservative, while the other half was preserved using Oragene™'s preservative. A portion of these samples were pooled together to represent a general Norgen-preserved saliva sample, and an Oragene™-preserved saliva sample. The rest of the samples were used to determine sample-tosample variation among both preservatives.

Saliva DNA extraction

DNA was extracted from all saliva samples using the Beckman Coulter Agencourt DNAdvance Kit, as per the manufacturer's protocol, with saliva-specific modifications made to the binding procedure. Briefly, saliva (both Norgen and Oragene) was incubated at 55°C for 1 hour, prior to DNAisolation. For Norgen Preserved Saliva, Bind1 Buffer was not used, and instead replaced by 10 µL of Norgen's proteinase K, and the sample was incubated for 20 minutes at 55°C. After the incubation, 340 µL of Bind2 Buffer (containing the magnetic beads) was added to the tube. The lysate was mixed by pipetting, followed by incubation on the magnetic rack for 8 minutes. The subsequent wash and elution steps were followed as per the manufacturer's protocol. Saliva DNA was eluted in 50 µl of Elution Buffer. For Oragane™-preserved saliva, the optimized protocol suggested by DNA Genotek (PD-PR-106) was followed. A Norgen-isolated control (spin column-based) was also used to compare yield and quality of Norgen-preserved saliva DNA isolated from the Norgen DNA Isolaton Kit (Cat# 45400) and the Agencourt DNAdvance kit, to confirm adaptability of the preservative to other systems.

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 Norgen-preserved and Oragene™-preserved saliva samples using the Beckman Coulter Agencourt DNAdvance Kit, a magnetic bead system. A Norgen spin column control was also used (with DNA isolated using the manufacturer's protocol) as a positive control, as the Norgen saliva preservative has been optimized for this kit. A pooled saliva sample was used to assess overall compatibility of both preservatives using theDNAdvance magnetic bead system. Fifteen microliters of 150 µL of Norgen's spin column.

elutions, and 4 µL of 40 µL of the DNAdvance elutions were run on 1X TAE 1.0% agarose gel (Figure 1). It was found that the DNA isolated from the Norgen-preserved saliva samples on the DNAdvance system was even cleaner than the Norgen spin column control (which the preservative was optimized for), and the DNA yield was higher than the Oragene™ preservative, which is the current leading market competitor. Next, to determine the quality of the DNA isolated using the DNAdvance magnetic bead system, 2 µL of purified DNA were used in a 20 µL qPCR reaction (SYBR Green®) using 5s rRNA primers (Figure 2). Based on the Ct values generated, the Norgen-preserved saliva and the Oragene™-preserved saliva were of comparable quality, with both samples amplifying at a Ct of ~20. The Spin Column control was found to have a slightly lower Ct value, despite the Norgen saliva preservative being optimized for this kit. This demonstrates the compatibility of Norgen's saliva preservative with the DNAdvance magnetic bead system.

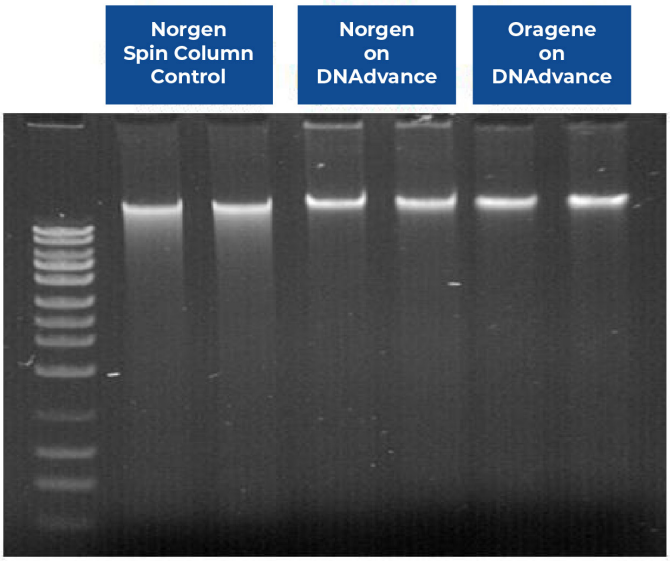


Figure 1. Comparison of DNA Yields from NorgenPreserved and Oragene™-Preserved Saliva on the DNAdvance System. Fifteen microlitres out of 150 µL of Norgen's spin column elution, and 4 µL out of 40 µL of the DNAdvance elutions were loaded on a 1X TAE 1.0% gel. The Norgen-preserved saliva sample appears to generate higher DNA yields than Oragene™-preserved saliva on the DNAdvance system, and the yield is also comparable to Norgen's spin column control.

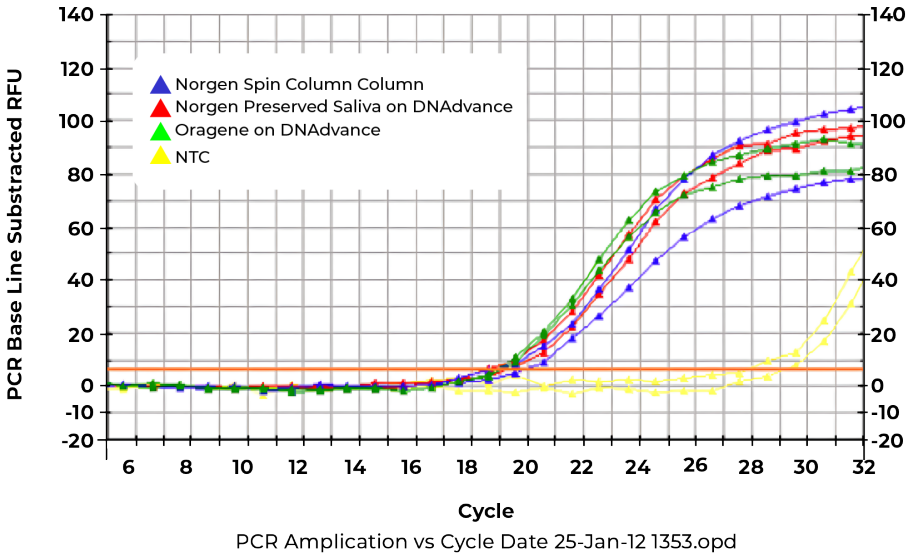


Figure 2. Comparison of qPCR Performance Using Norgen-Preserved and Oragene™-Preserved Saliva DNA on the DNAdvance System. Two microliters of DNA eluted from the DNAdvance elutions and Norgen's spin column control were used in a qPCR reaction with primers flanking the 5s rRNA gene. The Norgen-preserved and Oragene™preserved saliva DNA performed similarly using DNAdvance, indicating that the Norgen preservative (while optimized for the Norgen Saliva DNA Isolation Kit) is compatible with a magnetic bead system, producing high quality DNA using both systems (Norgen spin column and DNAdvance). Both Norgen and Oragene™ samples showed Ct values of ~20.

Individual saliva samples were also used to test both preservatives for sample-to-sample variations. Three individual saliva donors collected 2 mL of saliva, with half being preserved with Norgen's saliva preservative, and half being preserved with Oragene's™ saliva preservative. DNA was then isolated from all samples using the DNAdvance magnetic bead system. Four microliters (from 40 µL elutions) of purified DNA was then run on a 1X TAE 1.0% gel (Figure 3). Norgen's preservative was found to generate higher DNA yields than the Oragene™ preservative using the same saliva samples. This indicates that Norgen's preservative is more compatible with the DNAdvance magnetic bead system than our leading market competitor.

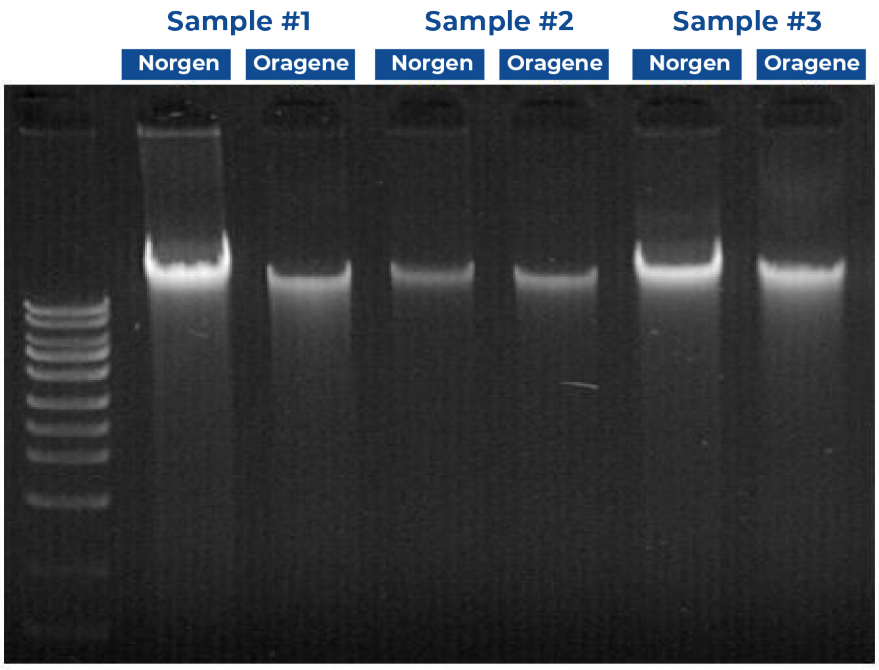
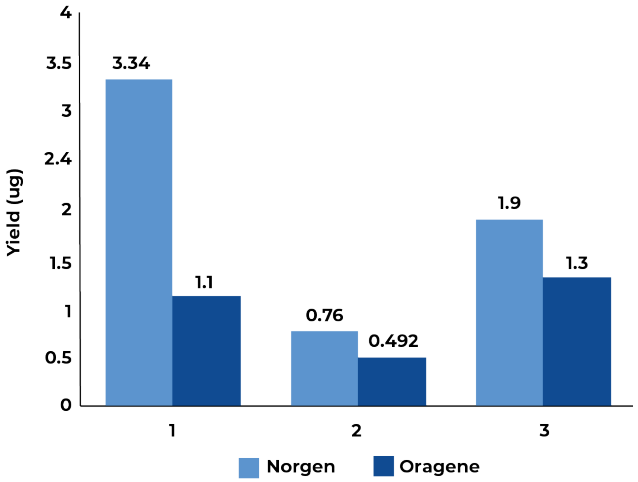


Figure 3. Comparison of DNA Yields from Three Different Saliva Donors Using Norgen's and Oragene's™ Saliva Preservative on the DNAdvance System. Four microliters out of 40 µL of DNAdvance elutions were loaded on a 1X TAE 1.0% gel. The Norgen-preserved saliva samples appear to generate higher DNA yields than Oragene™preserved saliva on the DNAdvance system, especially when the sample contains high amounts of DNA.

The saliva DNA purified from the samples in Figure 3 were then quantified using the NanoVue™ spectrophotometer, as per the manufacturer's protocol. The yield from each is depicted in Figure 4. In correlation with the gel, Norgenpreserved saliva performed exceptionally well using the DNAdvance system, generating higher yields than the same samples preserved using Oragene's™ preservative.

Also generated from the NanoVue™ spectrophotometer readings, the A260:A280 and A260:A230 ratios were used to assess the quality of DNA isolated from both preservatives, using the DNAdvance system. The average and standard deviation generated from the three samples was then calculated for both ratios. The average A260:A280 ratio (Figure 5) was found to be very similar between the Norgen and Oragene™-preserved saliva samples, however the average A260:A230 ratio (Figure 6) was found to be much higher for the Norgen-preserved saliva. These ratios are also very similar to the ratios generated from DNA isolated from Norgen-preserved saliva using the Norgen Saliva DNA Isolation Kit. These findings indicate that similar to Oragene™, Norgen's saliva DNA preservative is compatible with a magnetic bead system, such as the Agencourt DNAdvance Kit, offered by Beckman Coulter.



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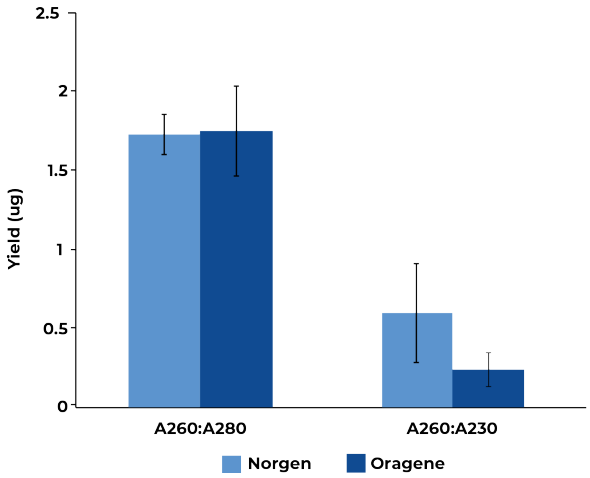

Figure 5. Comparison of DNA Quality from Norgen's and Oragene's™ Saliva Preservative on the DNAdvance System. Based on the three saliva samples used in Figures 3 and 4, the average A260:A280 and A260:A230 were calculated from reading given by the NanoVue™ Plus spectrophotometer. The Norgen and Oragene™ preservatives gave similar A260:A280 ratios using the DNAdvance system, while the A260:A230 generated using the Norgen-preserved saliva samples was much higher (more than double) that of the Oragene™-preserved saliva samples.

CONCLUSIONS

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

1. Norgen's Saliva DNA Preservative is Compatible with Magnetic Bead Systems. The Beckman Coulter Agencourt DNAdvance system was found to be fully compatible with Norgen's saliva DNA preservative, generating high quality DNA with comparable yields to the Norgen's Saliva DNA Isolation Kit (Cat# 45400).

2. Norgen's Saliva Preservative is Comparable to Oragene's™ Saliva Preservative on Magnetic Bead Systems. Norgen's saliva preservative is as compatible if not more suited for magnetic bead systems as Oragene's™ preservative, based on yield, quality and qPCR Ct values.

3. Norgen's Saliva DNA Preservative Optimally Performs across a Variety of Saliva Samples. Norgen's preservative produced higher quality and yields of DNA from different samples, compared to the leading competitor. Norgen's preservative outperformed Oragene's™ preservative on the DNAdvance system for both DNA yield across three different samples, as well as the average A260:A230. The average A260:A280 for both preservatives was similar.

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