Column-Based Isolation of DNA from Preserved Saliva Samples Offers High Quality, Consistent and Rapid DNA Isolation

APPLICATION NOTE #43

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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. In contrast to blood samples, saliva can be self-collected, is less costly to ship and is easier to store and process. Human genomic DNA extracted from buccal epithelial cells and white blood cells found in saliva can be used in various applications including diagnostic assays, epidemiological studies and surveys.

Saliva samples collected from different individuals have highly variable amounts of DNA. The amount of DNA present in saliva can vary between individuals based on a number of factors including health status, age, number of saliva samples collected, dehydration and the length of time that elapses during saliva collection. Furthermore, a number of factors may affect the quality of the DNA isolated from different saliva samples. For example, brushing and/or flossing teeth before saliva collection can introduce gum trauma that causes bleeding, and introducing blood into saliva can reduce the DNA quality. Also, chewing of some foods can introduce tiny cuts in the mouth which can increase the amount of blood cells present in the sample, therefore it is recommended that an individual does not eat for 30 minutes prior to providing a saliva sample. All of these different factors can affect the quality and yield of DNA isolated from saliva samples.

Norgen Biotek has developed a wide range of products that allow for the collection, preservation and isolation of DNA from saliva samples. In this application note, we compare the yield and quality of DNA isolated from preserved saliva samples using Norgen's **Saliva DNA Collection, Preservation and Isolation Kit (Cat # 35700),** which is based on ethanol precipitation against the yield and quality of DNA obtained using Norgen's Saliva DNA Isolation Kit (Cat# 45400), which is based on spin column chromatography. We compared the consistency of the isolation by having 2 independent scientists process the same samples and compared theoutcomes. Furthermore, we compared the methods in terms of speed and ease of use.

MATERIALS AND METHODS

DNA isolation

Saliva samples were collected from six healthy individual donors using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700). Collected saliva was mixed evenly with the preservative by vortexing and an equal volume of saliva mix was provided to two independent experimenters. The DNA was purified from the 0.5 mL of preserved saliva using the same Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700) as well as Norgen's Saliva DNA Isolation Kit (Cat# 45400; column based). Each independent experimenter used the same kits from the same lot.

Determination of DNA Yield and Quality

DNA yield and quality was determined using a NanoVue Plus TM spectrophotometer (GE Healthcare) according to the manufacturer's instructions. For visual analysis 10 µL of each 200 µL elution was also run on a 1.2% agarose gel.

Real-Time PCR Analysis

To analyze the purity of the DNA, 8 µ L of purified saliva DNA was directly added to 12 µ L of 2x PCR Master Mix reaction containing GAPDH primers without any dilution (F: 5'accacagtccatgccatcac3'; R:5'tccaccaccctgttgctgta3', 250 nM each. Amplicon size of 452bp) and SYBR Green I (Invitrogen Canada Inc. ON, Canada). Real-time PCR was performed on the iCycler iQ real-time system (Bio-Rad).

RESULTS AND DISCUSSION

Two independent experimenters isolated saliva DNA from 6 split samples using Norgen's ethanol precipitation-based Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700) as well as Norgen's column-based Saliva DNA Isolation Kit (Cat# 45400). The yield and quality of the isolated DNA was then compared using a NanoVue Plusspectrophotometer, as well as through visualization on an agarose gel. The results are summarized below.

	DNA Isolation Method	Average OD 260/280	Average Yield (ug)
Experimenter #1	Column-Based	1.93	1.9
	Ethanol Percipitation	1.65	5.2
Experimenter #2	Column-Based	1.92	1.7
	Ethanol Percipitation	1.66	4.3

Figure 1. Comparison of DNA Purity and Yield. The purity and yield of saliva DNA isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (ethanol-precipitation based isolation) and Norgen's Saliva DNA Isolation Kit (column-based isolation) from two independent experimenters is summarized.

From observing the results in Figure 1, it can be seen that the purity of DNA isolated using the spin columns was much higher than the purity of the DNA isolated using ethanol precipitation for both experimenters. Experimenter 1 obtained an average OD 260/280 of 1.93 when using the column-based method while Experimenter 2 obtained an average OD 260/280 of 1.92 using the same column-based method for DNA isolation. In contrast, the average OD 260/280 obtained by both experimenters using the ethanol precipitation-based method was 1.65 and 1.66. Therefore, based on the OD readings obtained, the spin-column method provided DNA with a much higher purity.

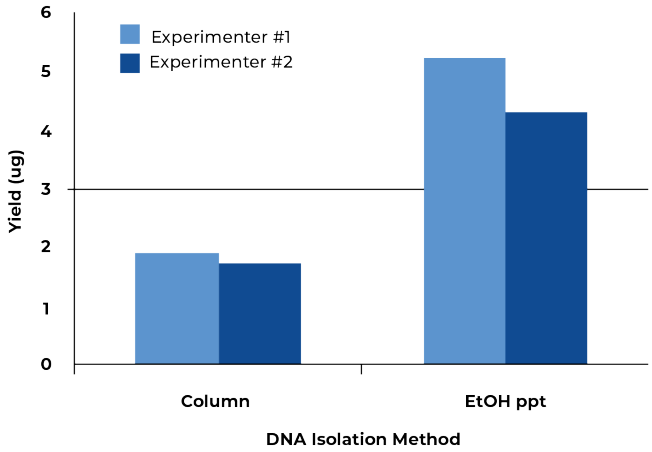

Figure 2. Variability in Yield between Two Different Experimenters. The graph shows the average yield obtained by each experimenter from the 6 saliva samples when using the column-based and ethanolprecipitation based methods for saliva DNA isolation.

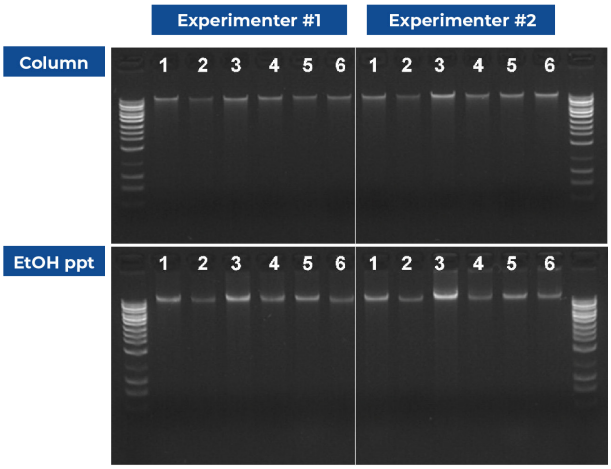

Figure 3. Variability in Saliva DNA Yield Between Experimenters. Saliva DNA was isolated from 6 saliva samples by two different experimenters using a column-based method and an ethanolprecipitation based method for saliva DNA isolation. The purified DNA (20 µL of each 200 µL elution) was run on a 1.2% agarose gel for visual analysis.

Figure 2 is a graph comparing the variability in yield when the ethanol precipitation-based DNA isolation method and the column-based DNA isolation method are performed by the two different experimenters. While the ethanol-based method resulted in higher yields of DNA, the method did show a much higher degree of variability between the 2 experimenters than the column-based method showed. The column-based saliva DNA isolation method resulted in only a 5% variability between the 2 experimenters while the ethanol precipitation-based method resulted in a variability of over 10%. The variability can be further seen in Figure 3, in which the eluted DNA was run on a 1.2% agarose gel for visualization. The gels comparing the different experimenters using the same method of isolation further show that a greater degree of variability can be seen with the ethanol precipitation-based method than with the column-based method.

Saliva DNA purified from one of the six donors using each of the methods from Experimenter 1 and Experimenter 2 was then used as the template in a real-time PCR reaction using GAPDH primers for detection. Twelve µL of the DNA from each elution was added directly to the Master Mix with no further dilution in order to test for the presence of any inhibitors. As it can be seen in Figure 3, the saliva DNA

isolated by both experimenters using both methods was successfully amplified, indicating that there are no inhibitors present and that the purified DNA can indeed be used successfully in downstream applications. Therefore both the column-based method and the ethanol precipitation-based method provide sufficient yields of high quality DNA for downstream applications.

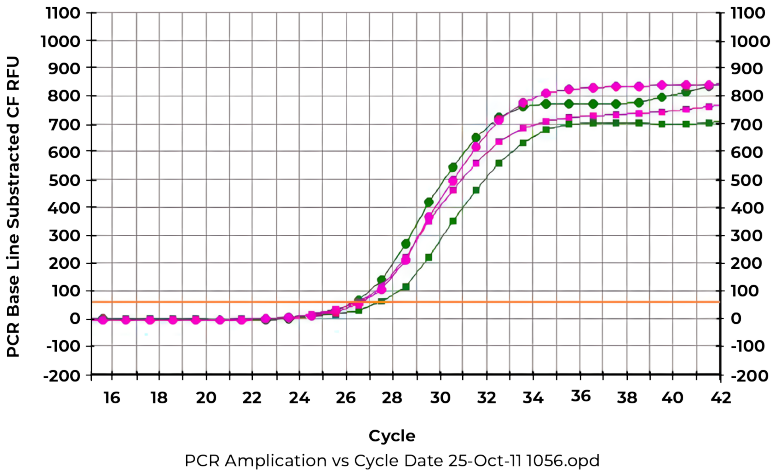

Figure 4. High Quality, Inhibitor-Free DNA. Saliva DNA isolated using both the spin column-based procedure and the ethanol precipitation-based procedure was successfully amplified in a real-time PCR reaction. The green lines correspond to Experimenter 1, the pink lines correspond to Experimenter 2, the round lines indicate columnpurified DNA and the square lines indicate ethanol precipitationpurified DNA.

The 2 independent experimenters also compared the spin column-based method and the ethanol precipitation-based method in terms of ease of use and time required to perform the procedure. Both experimenters found the column-based method provided by the Saliva DNA Isolation Kit to be a much more rapid and user-friendly method to perform. DNA isolation using the spin columns was completed in approximately 30 minutes while the isolation with ethanol precipitation required approximately 2 hours to complete.

CONCLUSION

Thus it can be seen that both the Saliva DNA Collection, Preservation and Isolation Kit (ethanol-based purification) and Norgen's Saliva DNA Isolation Kit (spin column-based purification) provide high quality DNA in sufficient yields to be used in various downstream applications. The spincolumn based method has the additional benefits of isolating DNA with a higher purity, as indicated by the OD 260/280 values of 1.92 and 1.93 obtained by both the experimenters. Furthermore, the spin-column basedmethod was more consistent then the ethanol-precipitation based method in terms of yield. In addition, the columnbased method can be performed in approximately 30 minutes, while the ethanol-precipitation method requires approximately 2 hours to complete.

Therefore, based on this limited study, it was found that the column-based method for saliva DNA isolation is rapid and user-friendly, and provides a more consistent yield of highquality, inhibitor-free DNA that can be used in numerous downstream applications.

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