High Efficiency Real-Time PCR from Saliva DNA

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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 user-friendly, all-in-one kit for the collection, preservation, storage and purification of DNA from saliva. Using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700), 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. Once received at the lab the samples can be stored for up to 2 years at room temperature, and high quality DNA can be isolated using the reagents provided with the kit.

Norgen's Saliva DNA Collection, Preservation and Isolation Kit provides high quality DNA that is compatible with most downstream applications. Real-time PCR is one major application that is used in quantification of gene copy numbers, viral quantitation, pathogen detection as well as array verification and genotyping. In this study DNA was collected and isolated from 50 donors using Norgen's Saliva DNA Collection, Preservation and Isolation Kit, and the DNA was used as the template in real-time PCR reactions to show the high quality of the isolated DNA.

MATERIALS AND METHODS

DNA Isolation

Saliva samples were collected from 50 donors by using Norgen's Saliva DNA Collection, Preservation and Isolation Kit according to the Kit instructions (Cat# 35700). Saliva DNA was then isolated from the 50 samples using 0.5 mL saliva-preservative mixture and by following the isolation protocol supplied with the kit.

Real-Time PCR Amplification

One hundred nanograms of each DNA saliva sample was then used as the template in a real-time PCR reaction using 2X SYBR Green/Fluorescein qPCR Master Mix (Norgen Biotek, Cat# 28109). GAPDH specific primers (F: 5'accacagtccatgccatcac3'; R:5'tccaccaccctgttgctgta3', 250 nM each. Amplicon size of 452bp) were used in the reaction amplified on the iCycler iQ real-time system (Bio-Rad).

RESULTS AND DISCUSSION

Saliva DNA must be pure and free from inhibitors in order for the DNA to be used in sensitive downstream applications. Here, DNA was collected and isolated from 50 saliva samples using Norgen's Saliva DNA Collection, Preservation and Isolation Kit, and was subsequently used as the template in Real-Time PCR reactions to detect the GAPDH gene. Figure 1 shows both the successful and consistent amplification of all 50 DNA samples (Panel A), and the melt curve indicates real amplification from all templates (Panel B). Figure 2 shows a graph of the Ct values from all the DNA samples, and the consistent values indicate that the DNA samples were all of a similar quality. Therefore, all 50 DNA samples were amplifiable on real-time PCR and the average Ct value was 17.56±0.61.

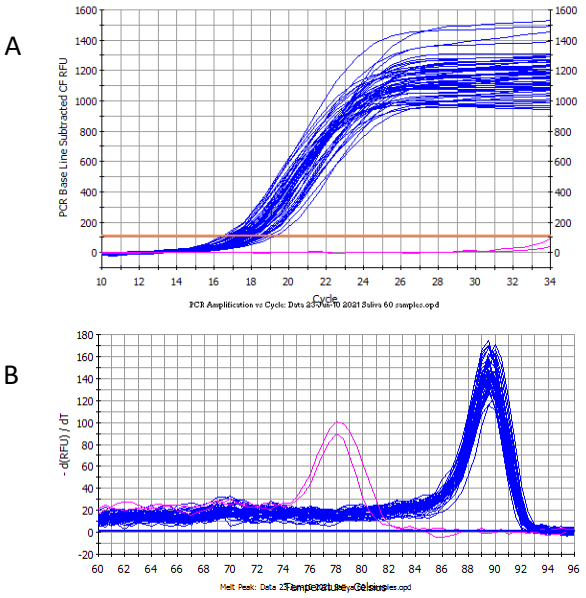

Figure 1. Amplification of the GAPDH Gene from 50 Saliva DNA samples. (A) Real-time PCR amplification and (B) melt-curve analysis. DNA samples are in blue color and the no template control is colored in pink. All the DNA templates were successfully amplified with high efficiency.

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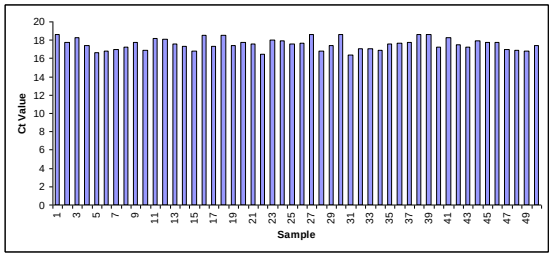

Figure 2: Ct values of the real-time amplification of GAPDH gene from the 50 saliva DNA samples. The average Ct value is 17.56±0.61.

CONCLUSION

DNA isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kit is of a very high quality, and is compatible with PCR applications, including real-time PCR. All 50 saliva DNA samples were amplified with similar Ct values, indicating the consistency of the DNA isolation.

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