Efficient Restriction Enzyme Digestion of Saliva DNA isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kits

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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 simple method for the collection, preservation, storage and purification of DNA from saliva using Norgen's Saliva DNA Collection and Preservation Devices (Cat. RU49000) and Norgen's Saliva DNA Isolation Kit (Cat. RU45400). 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 preservative 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. The DNA subsequently isolated from the preserved samples is of a high quality and can be used directly in sensitive downstream diagnostic assays such as real-time PCR. In this application note, 3 different saliva samples are collected using Norgen's Saliva DNA Collection and Preservation Devices (Cat. RU49000), and the DNA is subsequently isolated and digested to indicate the purity of the DNA.

METHODS AND MATERIALS

DNA Isolation

Three different saliva samples were collected using Saliva DNA Collection and Preservation Devices (Cat. RU49000). DNA was purified using Norgen's Saliva DNA Isolation Kit (Cat. RU45400) according to the supplied protocol.

Restriction Enzyme Digestion and Inactivation

HindIII (New England Biolabs) digestion was carried out according to manufacturer's instructions. One microgram DNA isolated from saliva samples was digested using 10 units of HindIII in a 20 µL reaction and incubated for 90 min at 37°C. The enzyme was heat-inactivated at 70°C for 20 min.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to visualize the HindIII-digested saliva DNA. Generally, 50% of the digested DNA was run on a 1.2% agarose gel in Tris-Acetate-EDTA buffer at 150V. The gel was then viewed under ultraviolet (UV) light and the image was captured using AlphaImager 2200 (Alpha Innotech).

RESULTS AND DISCUSSION

Purified Saliva DNA must be pure and free from inhibitors in order for the DNA to be used in sensitive downstream applications. Here, 3 samples of Saliva DNA was collected using Saliva DNA Collection and Preservation Devices (Cat. RU49000) and isolated using Norgen's Saliva DNA Isolation Kit (Cat. RU45400), and was subsequently digested using HindIII. Figure 1 shows the results of the HindIII digestion of saliva DNA from three different saliva samples. The digested (D) and the un-digested (U) DNA from each sample were run side by side to show quality of the saliva DNA before and after HindIII-digestion.

CONCLUSIONS

The results shows that the saliva DNA isolated using Norgen's Saliva DNA Isolation Kit (Cat. RU45400) is of a high quality and is compatible with restriction enzyme digestion. Restriction enzyme digestion is critical for many genomic downstream applications, therefore the purified DNA must be of a high quality and free of inhibitors such that it is amenable to digestion.

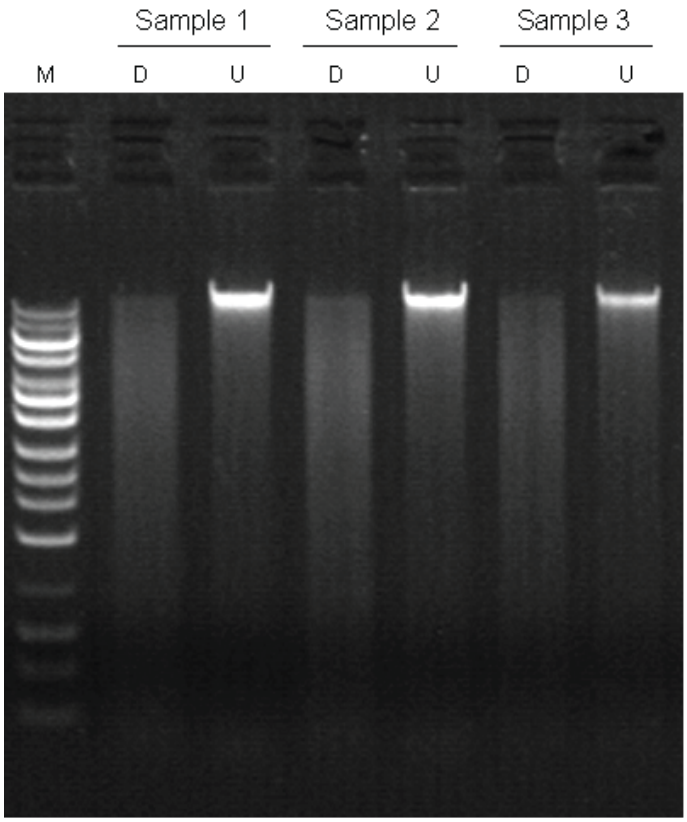

Figure 1. A 1.2% agarose gels showing the HindIII digestion of three different saliva samples. U=Undigested, D=Digested and M= Norgen's UltraRanger 1 kb DNA Ladder (Cat. 12100).

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Saliva DNA Collection and Preservation Devices (50)	50 Devices	RU49000
Saliva DNA Isolation Kit	50 Preps	RU45400

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