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

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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 Individual Saliva DNA Collection and Preservation Devices (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 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, Preservation and Isolation Kit, and the DNA is subsequently isolated and digested to indicate the purity of the DNA.

MATERIALS AND METHODS

DNA Isolation

Three different saliva samples were collected using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700). DNA was purified 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, Fifty percent 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 Alphalmager 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, DNA was collected and isolated from 3 saliva samples using Norgen's Saliva DNA Collection, Preservation and Isolation Kit, 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.

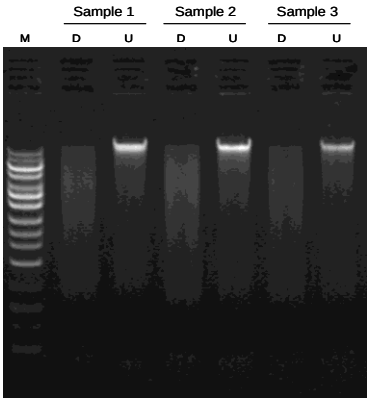

Figure 1. A 1.2% agarose gels showing the HindIII digestion of three different saliva samples. U=Undigested, D=Digested and M= Norgen's UltraRanger 1 kb DNA Ladder (Cat# 12100).

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CONCLUSION

The results shows that the saliva DNA isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kit 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 of a high quality and free of inhibitors such that it is amenable to digestion.

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