Sex Determination from 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. 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. 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 PCR. In this application note 22 different saliva samples were collected from 14 male individuals and 8 female individuals using Norgen's Saliva DNA Collection, Preservation and Isolation Kit. The DNA is subsequently isolated and sex (male or female) is determined via nested PCR.

METHODS AND MATERIALS

DNA Isolation

Twenty two different saliva samples were collected from 14 male individuals and 8 female individuals using Norgen's Saliva DNA Collection, Preservation and Isolation Kit (Cat# 35700). DNA was purified according to the supplied protocol.

Sex Determination using nested PCR

One hundred nanograms from each sample was used as a template for a nested PCR amplification of the Ychromosome using Norgen's 2X PCR Master Mix (Cat# 28007). Amplification of the housekeeping gene GAPDH was used as a control. Table 1 shows the Y-chromosome and the GAPDH specific primers as well as the expected amplicon sizes. The PCR amplification was performed on the iCycler iQ real-time system (Bio-Rad).

Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to visualize the amplified DNA resulting from the nested PCR amplification of the Y-chromosome, as well as the GAPDH control. Generally, 50% of the digested DNA was run on a 1.5% 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).

Primer	Sequence	Amplicon Size (bp)
Y1 (Forward)	5'-TCCACTTTATTCCA GGCCTGTCC-3'	154
Y1 (Reverse)	5'-TTGAATGGAATGGG AACGAATGG-3'	
Y2 (Forward)	5'-GTCCATTACACTACATTCCC-3'	77
Y2 (Reverse)	5'-AATGCAAGCGAAAGGAAAGG-3'	
GAPHD (Forward)	5'-ACCACAGTCCATGCCATCAC-3'	452
GAPHD (Reverse)	5'-TCCACCACCCTGTTGCTGTA-3'	

Table 1. Primer Sequence

RESULTS AND DISCUSSION

Figure 1 shows the nested PCR amplification of the Ychromosome as well as the GAPDH control. The nested PCR amplification of the Y-chromosome clearly showed the 154bp primary amplicon and the 77bp secondary amplicon in all male samples. None of the 8 female samples showed either the 154bp or the 77bp Y-chromosome amplicons. GAPDH was successfully amplified in all samples indicating the absence of any PCR inhibition for the 22 samples, further verifying that the absence of amplification in the 8 female samples was not due to inhibition.

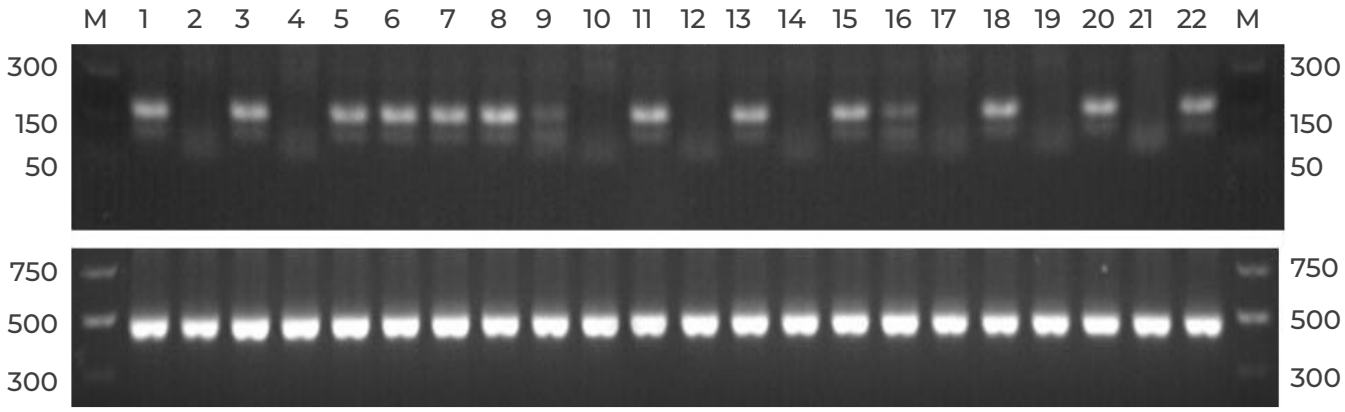

Figure 1. A 1.5% agarose gels showing the nested PCR amplification of the Y-chromosome and the GAPDH among 22 different saliva samples. M is Norgen's FastRunner DNA Ladder (Cat# 12800).

CONCLUSIONS

Saliva DNA isolated using Norgen's Saliva DNA Collection, Preservation and Isolation Kit is of a high quality and can be used for as the template in nested PCR reactions for sex determination and other similar applications.

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