Stability of DNA Stored in Norgen's Saliva DNA Preservative for 52 Months at Room Temperature

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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. The isolation of high quality DNA from saliva is not without its problems however. The number of DNA-containing cells found in saliva can vary significantly from individual to individual. Adequate amounts of saliva must therefore be collected to ensure that DNA can be extracted in an amount sufficient for testing. As enzymes that degrade DNA are found in saliva, methods must also be employed to protect the DNA in the sample. For currently available collection devices which do not employ preservative this requires that the saliva be collected into approved cryovials, held on ice and then frozen at -20°C as soon as possible. The addition of preservative to the collection vials eliminates the need to immediately process or freeze the saliva samples and allows the samples to be shipped at ambient temperature.

Norgen Biotek Corp. has developed a Saliva DNA Preservative which allows for the long-term preservation of saliva samples at ambient room temperature, making this buffer ideal for saliva storage and shipping. This buffer is available as a product on its own, and is also included with our different saliva DNA collection, preservation, storage and purification devices and kits. The Saliva DNA 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, the long-term stability of saliva DNA in Norgen's Saliva DNA Preservative is analyzed. Preserved saliva samples were stored at room temperature for up to 52 months, and the DNA was subsequently isolated at various time points and analyzed.

MATERIALS AND METHODS

DNA Isolation

Saliva samples were collected from numerous donors and mixed, and then an equal volume of Norgen's Saliva DNA Preservative was added to the saliva. Equal aliquots of the preserved DNA saliva samples were stored at room temperature for 52 months. Saliva DNA was subsequently isolated at various time points (1 week, 3 weeks, 2 months, 4 months, 6 months, 10 months, 12 months, 20 months, 32 months, 40 months and 52 months) from 0.5 mL of the saliva/preservative sample using Norgen's Saliva DNA Isolation Kit (Cat# 45400) as per the recommended protocol.

Gel electrophoresis

For visual analysis 10 µL of DNA from the final DNA elution was loaded on to a 1% agarose TAE gel and run for 25 minutes at 150 V. The gel photo was taken using an AlphaImager™ IS-2200 (Alpha Innotech).

PCR Amplification

The purified DNA was then used as the template in a real-time PCR reaction. Briefly, 2 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture (TaqMan probe) containing 2.5 mM GAPDH primer pair. The PCR samples were amplified under the real-time program; 95°C for 5 minutes for an initial denaturation, 50 cycles of 95°C for 15 second for denaturation and 60°C for 30 seconds for annealing and extension. The reaction was run on a CFX 96 real-time system (Bio-Rad).

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RESULTS AND DISCUSSION

Saliva samples often need to be stored for a period of time prior to DNA isolation and analysis. Traditionally saliva samples are held on ice and then frozen at -20°C as soon as possible, however such storage leads to increased costs and is not always convenient, especially in resource-limited settings. The ability to safely store saliva samples at ambient temperatures without any degradation of the DNA is ideal. Here saliva samples were collected from numerous donors and mixed, and then an equal volume of Norgen's Saliva DNA Preservative was added to the saliva. The preserved saliva was then stored at room temperature for up to 52 months. Saliva DNA was subsequently isolated at 1 week, 3 weeks, 2 months, 4 months, 6 months, 10 months, 12 months, 20 months, 32 months, 40 months and 52 months from 0.5 mL of the saliva/preservative sample using Norgen's Saliva DNA Isolation Kit (Cat# 45400). For visual analysis, 10 µL of the purified DNA was run on an 1.2% agarose TAE gel.

As it can be seen, there is no evidence of DNA degradation after the saliva samples are stored for 52 months at ambient temperatures in Norgen's Saliva DNA Preservative (Figure 1). Furthermore, the size of the DNA was maintained at over 24 kb for the entire 52 month period. An improved DNA yield was observed from the 20 month collection point onward, due to improvements made to the saliva DNA isolation method.

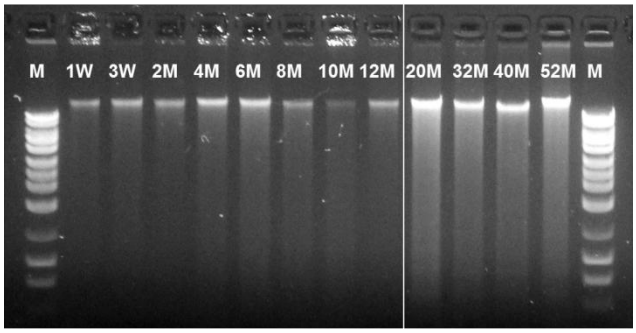

Figure 1. Stability of DNA Preserved in Norgen's Saliva DNA Preservative at Room Temperature for up to 52 Months. Lane M: Norgen's UltraRanger 1 Kb DNA Ladder. (Cat#12100).

Next, 20 ng of the purified DNA was used as the template in a TaqMan real-time PCR reaction to detect the GAPDH gene, and the Ct values were graphed. As it can be seen in Figure 2, the DNA isolated from the saliva samples stored at room temperature from 1 week up to 52 months could all be successfully amplified using real time PCR.

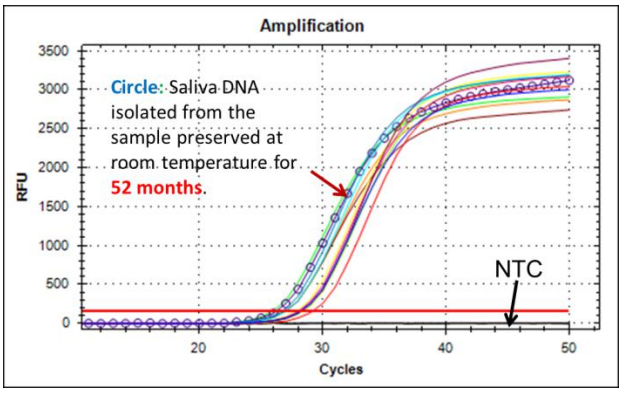

Figure 2. Real Time PCR Amplification of Saliva DNA from the saliva samples stored at room temperature from 1 week up to 52 months (12 time points). All isolated saliva DNA samples were successfully amplified without the sign of the PCR inhibition. NTC: no template control.

Furthermore, the quality of the saliva DNA stored at room temperature did not change from 1 week to 52 months, as indicated by the fact that the Ct values remained consistent **(Figure 3)**.

The improvement in the Ct values from the real-time PCR graph above also indicate the impact of the improved saliva DNA isolation method provided by the Saliva DNA Isolation Kit (Cat# 45400).

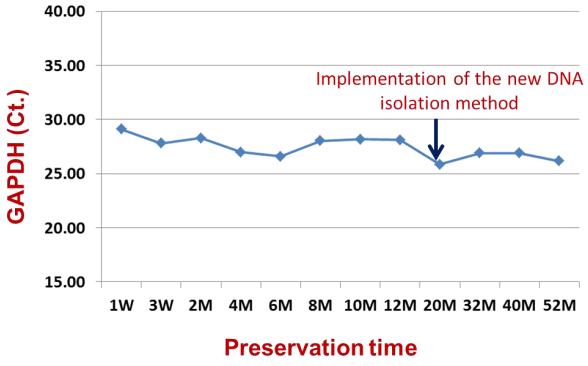

Figure 3. Detection of GAPDH using TaqMan real-time PCR from the saliva samples stored at room temperature from 1 week up to 52 months.

CONCLUSIONS

1. Saliva DNA can be preserved in Norgen's Saliva DNA Preservative for up to 52 months at room temperature.
2. The full size range of the preserved saliva DNA is maintained for 52 months at room temperature.

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3. The purified DNA can be successfully amplified in sensitive downstream applications such as TaqMan real-time PCR.

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