

Stability of DNA Stored in Norgen's Saliva DNA Preservative for Over 30 Months

W. Kim, PhD¹, M. El-Mogy, PhD¹, and Y. Haj-Ahmad, Ph.D^{1,2}

¹Norgen Biotek Corporation, Thorold, Ontario, Canada

²Centre for Biotechnology, Brock University, St. Catharines, Ontario, Canada

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 4°C and room temperature for up to 32 months, and the DNA was subsequently isolated 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 4°C and room temperature for 32 months. After the 32 months, saliva DNA was subsequently isolated from the saliva/preservative sample in duplicate 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 Alphamager™ 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 (SYBR Green) 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, 40 cycles of 95°C for 15 second for denaturation and 60°C for annealing and extension. The reaction was run on an iCycler iQ real-time system (Bio-Rad).

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 are mixed with Norgen's Saliva DNA Preservative and stored at 4°C and room temperature for up to 32 months. Saliva DNA was isolated from the saliva samples stored at the different temperatures in duplicate after 32 months. The saliva DNA isolated from the saliva samples stored at 4°C and room temperature was subsequently run on a 1% agarose TAE gel for visual analysis. As it can be seen in Figure 1, there is no evidence of DNA degradation after the saliva samples are stored for 32 months at ambient temperatures in Norgen's Saliva DNA Preservative. Furthermore, the size of the DNA was maintained over 24 kb for the entire 32 month period.

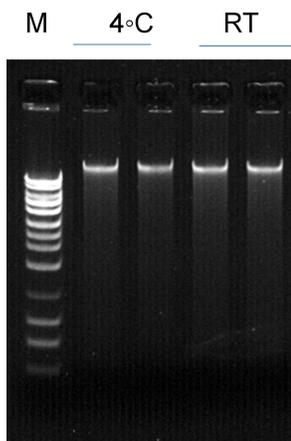


Figure 1. Stability of DNA preserved in Norgen's Saliva DNA Preservative at ambient temperature for up to 32 months. M: Norgen's UltraRanger 1 Kb DNA Ladder. (Cat#12100).

These DNA samples were then used as the template in a real-time PCR reaction to detect the GAPDH gene. DNA samples must be of a high quality in order to be used successfully in downstream amplification reactions. As it can be seen in Figure 2, the DNA isolated from the saliva samples stored at 4°C and room temperature for 32 months could all be successfully amplified using real time PCR.

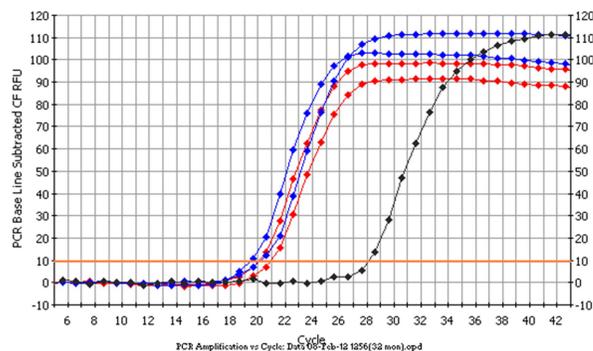


Figure 2. Real-Time PCR detecting GAPDH gene from saliva DNA isolated from Norgen-preserved saliva stored at 4°C (red lines) and room temperature (blue lines) for 32 months. The purified DNA was of a high quality and could be used in sensitive downstream applications.

The Ct values from the real-time PCR above are summarized in Table 1. As it can be seen, the Ct values obtained from the saliva samples stored at 4°C and room temperature were the same, indicating that the quality of the saliva DNA stored at the 2 temperatures did not change over the 32 months of storage.

Table 1. Ct Values from the Real Time PCR Amplification of GAPDH using Saliva DNA isolated from Norgen-Preserved Saliva after 32 Months Storage at 4°C and Room Temperature.

Target	Temp.	Ct.	Avg. Ct.	Stdev
GAPDH	4°C (Red)	21.1	21.4	0.3
		21.7		
	RT (Blue)	20.4	21	0.6
		21.6		
	NTC	29	29	0

CONCLUSION

High quality saliva DNA can be isolated and successfully amplified from saliva samples stored in Norgen's DNA Preservative at 4°C and room temperature for 32 months.