Stabilization of DNA from Saliva at Ambient Temperature

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Biomatrica
THE BIOSTABILITY COMPANY

ABSTRACT

Saliva provides an alternative to blood as a biological fluid in diagnostic applications. The major advantages of saliva over other biological fluids is the fact that it is non-invasive and can be collected with very limited training. Biomatrica has developed a novel formulation that preserves the integrity of genomic DNA at room temperature as well as elevated temperature for up to 1 year. This makes the saliva/preservative mixture ideal for storage and shipping instead of using expensive freezers and risking samples in cold packages during shipment.

Biomatrica's DNAgard Saliva (DGS) is based on our innovative technology platform applied to a chemical design of a long-term saliva preservative that protects DNA in saliva with high yield and quality comparable to cold-stored samples but at ambient temperatures.

OBJECTIVES

The goals of this work are to:

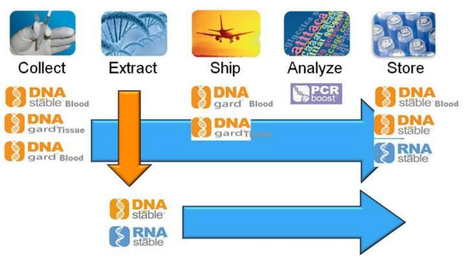
a) Evaluate the utility of a new saliva preservative – DNAgard Saliva – to protect DNA in saliva at ambient or elevated temperatures.

b) Demonstrate the compatibility of DNAgard Saliva with different isolation methods.

c) Evaluate any potential interference of DNAgard Saliva in down stream applications such as PCR.

"GARD" STABILIZATION TECHNOLOGIES

GARD® - Technology: Liquid Stability



Biomatrica has extended its biostabilization technology to liquid-based stabilization of nucleic acids ("Gard" products) using novel types of biostability molecules. Nucleic acid preservation starts as soon as the biological sample contacts the liquid stabilizer. The stabilizing molecules disrupt the cellular membranes, penetrate immediately the other cellular structures and inhibit nuclease activity as well as degradation through free radicals. In addition the stabilizers protect the nucleic acids in the biosample specimen from hydrolysis such as depurination.

MATERIALS AND METHODS

Sample collection: Saliva samples were collected from 5 different donors. Individual donors were asked to fast for at least a 30min period. After fasting, donors were required to wash their mouths with water to remove any food, wait an additional 10minutes before depositing saliva. Saliva collection was completed within 15minutes. The collected saliva was immediately mixed with DNAgard Saliva in two ratios: 1:1 (v:v) or 3:4 (v:v) formulation to saliva ratio.

DNA Isolation: DNA was isolated using 3 different techniques: (1) column extraction with the QIAamp DNA mini kit (QIAGEN), Organic[1] (phenol/chloroform) extraction, and a precipitation[2] method.

Analysis: DNA integrity was analyzed on a 0.8% agarose gel and 10% of the eluted DNA was loaded to the gel. DNA yield was determined by fluorescence using the Quant-iT™ PicoGreen dsDNA Assay Kit (Invitrogen).

RESULTS

Saliva samples from 4 donors stored in DNAgard Saliva for 31/2 months at room temperature

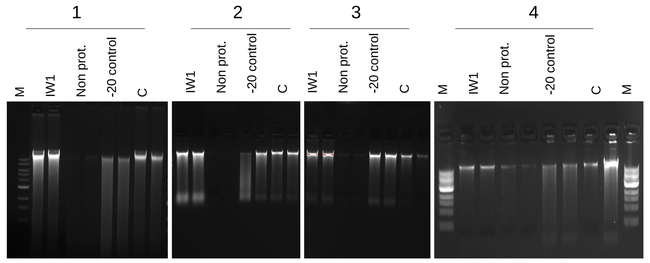


Figure 2. DNAgard Saliva protects DNA in saliva for several months. 200µL 4 saliva donor samples were mixed with DNAgard Saliva (150µL) and stored at room temperature for over 3months. As negative and positive controls, 200µL of saliva was stored at room temperature and -20 C freezer, respectively. For all 4 samples, DNAgard Saliva gave higher DNA yield compared to samples preserved in Oragene•DNA (C) or at -20 C (control). Marker, M is 1kb DNA ladder.

DNA yield in µg/mL

Donors	DGS	NP	-20C	OrD
1	36.0	1.2	19.9	17.0
2	26.1	0.2	13.0	10.8
3	12.7	0.6	13.7	8.8
4	1.8	0.7	2.7	1.8

Table 1. DNA was quantified using a sensitive fluorescence assay method, picogreen. DGS = DNAgard Saliva; NP = non-protected saliva at room temperature, -20C (freezer control); and OrD = Oragene•DNA.

Downstream application after 3½ months storage at room temperature

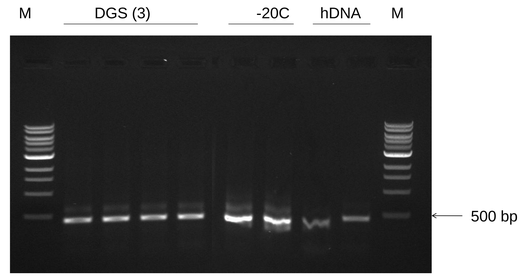
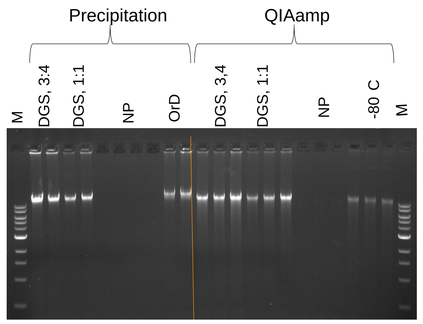


Figure 3. Representative end point PCR of purified DNA after 3½ months storage at room temperature. PCR amplification of 500 bp human β-actin was preformed on ~ 25 ng of DNA isolated using QIAamp from donor 3 saliva/preservative samples stored at room temperature and positive control saliva stored at -20 C for 3½ months.

DNAgard Saliva compatibility with different isolation methods



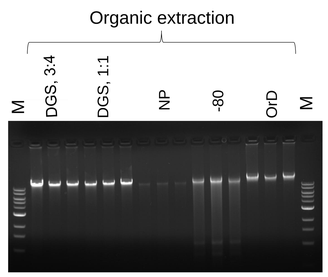


Figure 4. DNAgard Saliva was mixed with saliva (200µL) in 3:4 (v:v) and 1:1 (v:v) and stored at 50 C. Non protected (NP) and positive control samples of the same volume of saliva (200µL) were stored at 50 C and -80 C, respectively. After incubation for 18days, DNA was isolated using three isolation methods: EtOH precipitation, QIAamp and PhOH/$ChCl_3$ extraction. According to picogreen DNA quantitation, the precipitation and organic extraction methods afforded a little higher DNA yield than the column based QIAamp method.

PCR following DNA isolation after 18 days storage at 50 C

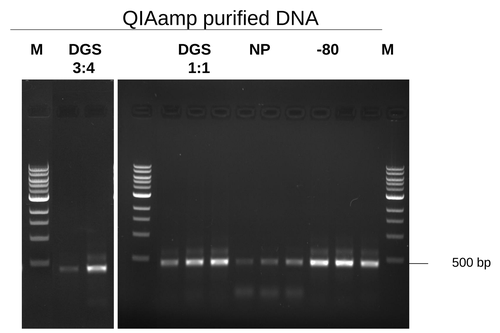


Figure 5. Representative end point PCR of purified DNA after 18 days storage at 50 C. PCR amplification of 500bp human β-actin was performed on ~25ng of DNA isolated using QIAamp from DNAgard Saliva samples stored at 50 C, non-protected saliva stored at 50 C and positive control saliva stored at -80 C 18 days. M is a 1kb DNA ladder.

Accelerated aging testing - DNA in saliva preserved for 63 days at 50 C in DNAgard Saliva.

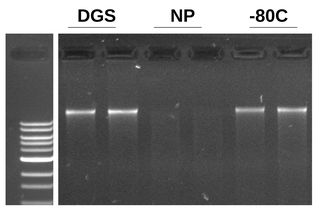


Figure 6. DNAgard Saliva protects DNA in saliva for more than two months at elevated temperature. 200µL of saliva was mixed with DNAgard Saliva (150µL) and stored at 50 C for over 2 months. As negative and positive controls, 200 µL of saliva was stored at room temperature and -80 C freezer, respectively.

CONCLUSIONS

1. DNAgard Saliva preserves the integrity of DNA in saliva for several months at ambient temperature. It also prevents DNA degradation at elevated temperature which may occur during sample shipment.
2. DNAgard Saliva formulation generally provided higher DNA recovery and quality compared to freezer control samples.
3. Genomic DNA isolated from DNAgard Saliva-preserved sample can be isolated with most common purification methods, and the purified DNA has been shown to be compatible with downstream applications.
4. Accelerated aging experiments (not shown here) have demonstrated that genomic DNA from DNAgard Saliva is stable for more than one year.