DNA purification from saliva stabilized in DNAgard® Saliva using an organic extraction method

Senait Ghirmai, Ph.D

Introduction

DNAgard® Saliva (Cat No. 97021-011A) allows for the efficient collection, preservation, shipping and storage of saliva samples for DNA purification and analysis. The product is designed for ease of use, with a simple process from collection to storage. DNA in saliva samples are preserved for up to 2 years at room temperature, with high quality DNA recovery. The stabilization offers flexible DNA recovery from a wide range of DNA purification kits. In this study, we show DNA purification from human saliva samples stabilized in DNAgard Saliva using a phenol/chloroform extraction method.

Materials and Methods

Saliva sample processing and DNA purification: Saliva was collected from a single donor. 2 mL of saliva was mixed with DNAgard Saliva (1.5 mL) or a competitor's saliva stabilizer (2 mL) according to the manufacturers' protocols. The saliva samples were stored at 50°C for 18 days prior to DNA purification. Saliva without any stabilizer was stored at 50°C for the same period of time as a negative control. Saliva without stabilizer was stored at -80°C as a positive control. All samples were purified and analyzed in triplicate. DNA was purified using a phenol/chloroform extraction method. Below is the DNA purification protocol for a 350 µL aliquot of DNAgard Saliva stabilizer-sample mixture.

Cell lysis/protein digestion

- Digest with 163 µL of lysis buffer (10 mM Tris, pH 8.0; 10 mM EDTA; 0.1 M NaCl; 2% SDS) followed by addition of 8.2 µL Proteinase K (20 mg/mL, Cat No. AM2548, Invitrogen).
- Incubate at 56°C for 10 minutes. Centrifuge briefly.

Phenol/chloroform extraction

- Add an equal volume of tris saturated phenol-chloroform-isoamyl alcohol. Vortex briefly and spin for 2 minutes at 12,000 rpm at 4°C.
- Transfer supernatant (upper layer) to a new tube (avoid aspiration of the interlayer or organic phase).
- Add an equal volume of chloroform. Vortex and spin 2 minutes at 12,000 rpm at 4°C.
- Transfer supernatant (upper layer) to a new tube (avoid aspiration of the interlayer or organic phase).

DNA precipitation

- Add 35 µL 3M sodium acetate to the supernatant.
- Add 700 µL 95-100% Ethanol. Vortex briefly.
- Precipitate at -80°C for 1 hour or -20°C overnight.
- Spin 20 minutes at 12,000 rpm at 4°C.
- Carefully aspirate supernatant (do not lose the DNA pellet).

Ethanol wash

- Carefully add 1 mL 70% EtOH (do not vortex).
- Spin 10 minutes at 12,000 rpm at 4°C.
- Carefully aspirate supernatant (do not lose DNA pellet).
- Air dry the DNA pellet for 10 minutes at room temperature (do not overdry).

DNA Hydration

- Dissolve the DNA pellet in 100 µL TE buffer.

DNA analysis: DNA from each sample was analyzed using gel electrophoresis and quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit (Cat No. P11496, Invitrogen).

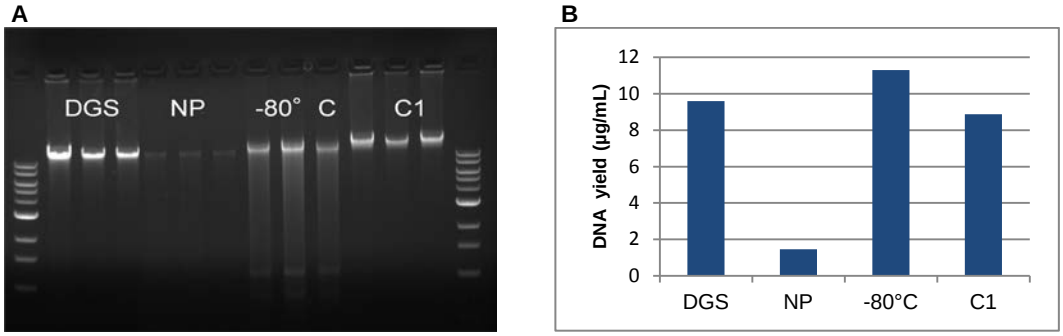

Figure 1. DNA image and quantification from saliva stabilized with DNAgard Saliva and other methods. The saliva samples stabilized with DNAgard Saliva (DGS) or a competitor's stabilizer (C1) were stored at 50°C for 18 days. Saliva without any stabilizer was stored at 50°C for the same period of time as a negative control (NP). A positive control was saliva stored in a -80°C freezer (-80°C). All samples were purified and analyzed in triplicate. DNA was purified as described above, and 10% of DNA solution was loaded on to a 0.8% agarose gel stained with ethidium bromide **(A)**. DNA was quantified by Quant-iT™ PicoGreen® dsDNA Assay Kit **(B).**

Results and Summary

In this study, we compared DNA quality and quantity from saliva samples stabilized by DNAgard Saliva and other methods. Our results demonstrate that the organic extraction method can be successfully applied for DNA purification from the saliva samples stabilized by DNAgard Saliva. In addition, we show that the DNA quality from DNAgard Saliva samples is better than positive controls and the competitor's stabilizer. In summary, DNAgard Saliva showed high DNA recovery and high DNA quality in human saliva samples using a phenol/chloroform extraction method. It provides an efficient way for collection and transport of saliva specimens at room temperature as well as elevated temperatures.

***Note**: Please read all instructions for the DNAgard Saliva prior to using this protocol.*

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