

DNA purification from saliva stabilized in DNAGard® Saliva using the Gentra® Puregene® Kit

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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 the Gentra® Puregene® Purification Kit (QIAGEN).

Materials and Methods

Saliva Sample Processing and DNA Purification: Saliva was collected from four adult donors. 2 mL of saliva was mixed with 1.5 mL DNAGard Saliva stabilizer. The stabilizer-saliva mixtures were then stored at 50°C for 5 days prior to DNA purification. All samples were purified and analyzed in triplicate. DNA was isolated using the Gentra Puregene Blood Kit (Cat No. 158467, QIAGEN). Below is the DNA purification protocol for a 200 µL aliquot of stabilizer-saliva mixture.

Cell Lysis/Protein Digestion

- 1 Mix the stabilizer-saliva mixture with 500 µL cell lysis buffer by pipetting up/down a few times.
- 2 Add 3 µL Proteinase K (20 mg/mL, Cat No. AM2548, Invitrogen) and mix by inversion.
- 3 Incubate at 55°C for 1 hour. Centrifuge briefly.
- 4 Add 3 µL Gentra RNase A solution (4 mg/mL) and mix by inverting 25 times.
- 5 Incubate for 15 minutes at 37°C and then 2 minutes on ice to quickly cool the sample.

Protein Precipitation

- 6 Add 200 µL Gentra protein precipitation solution. Vortex for 20 seconds at high speed.
- 7 Incubate for 5 minutes on ice, and centrifuge for 3 minutes at 13,000 x g.
- 8 Transfer the clear supernatant to a clean tube by pipetting. If the supernatant is not clear, repeat Step 7.

DNA Precipitation

- 9 Add 600 µL Isopropanol (100%) and 3 µL Gentra Glycogen solution (20 mg/mL).
- 10 Mix by inverting the sample 50 times.
- 11 Incubate the sample at room temperature for 5 minutes.
- 12 Centrifuge for 5 minutes at maximum speed.
- 13 Carefully discard the supernatant by draining on a clean piece of absorbent paper. Make sure that the pellet remains in the tube.
- 14 Add 600 µL 70% ethanol and invert several times to wash the DNA pellet.
- 15 Centrifuge at 13,000 x g for 1 minute.
- 16 Carefully discard supernatant and drain the tube on a clean piece of absorbent paper. Make sure that the pellet remains in the tube.
- 17 Allow sample to air dry for 5-10 minutes.

DNA Hydration

- 18 Add 100 µL TE buffer.
- 19 Rehydrate DNA by incubating at 65°C for 1 hour or over night at room temperature.

DNA Analysis: DNA from each sample was analyzed using gel electrophoresis and quantified using absorbance at 260 nm after background subtraction. See Figure 1 below.

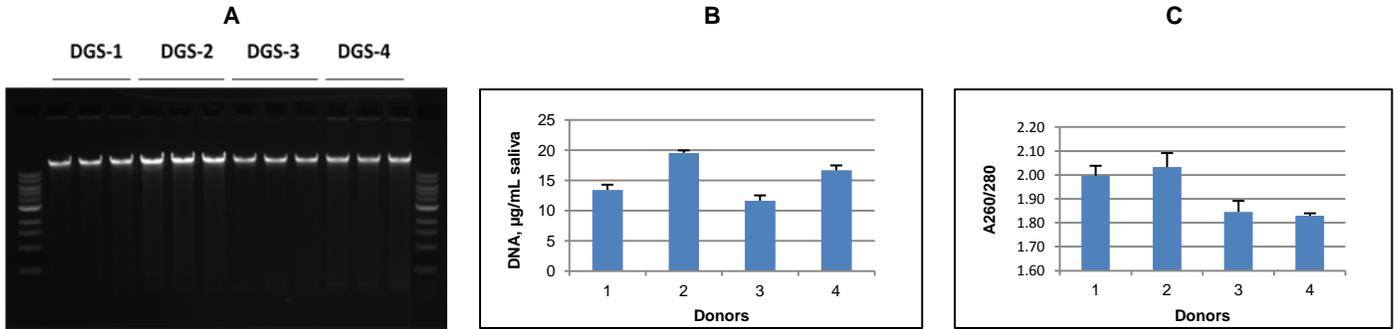


Figure 1. DNA quantities and qualities of saliva samples stabilized with DNAgard Saliva. The saliva samples from four donors stabilized with DNAgard Saliva (DGS) were stored at 50°C for 5 days. DNA purification is described as above. All samples were purified and analyzed in triplicate. **(A)** 10% of DNA solution was loaded on to a 0.8% agarose gel stained with ethidium bromide. **(B)** DNA was quantified by 260 nm absorbance after background subtraction. **(C)** DNA purity was determined by ratio of 260/280 nm absorbance.

Results and Summary

In this study, we show that saliva DNA stabilized with DNAgard Saliva reagent can be purified using Genra Puregene Purification Kit. This method provides both high DNA recovery and high DNA purity. Our results demonstrate that DNAgard Saliva reagent provides a safe and efficient way for collection and transport of saliva specimens at elevated temperature.

Note: Please read all instructions for the [DNAgard Saliva](#) prior to using this protocol.

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