Promega

Product Application

Automated purification of DNA from GeneFiX™ Saliva DNA Collection Devices

Purify DNA from human saliva collected in GeneFiX™ Saliva DNA Collection Devices using the Maxwell® RSC instrument and Maxwell® RSC Stabilized Saliva DNA Kit.

Kit: Maxwell® RSC Stabilized Saliva DNA Kit (Cat.# AS1630)

Analyses: UV absorbance, Dye-based quantitation and qPCR

Sample Type(s): Human saliva collected in GeneFix™ Saliva DNA Collection Device

Input: 1ml of total sample (500µl saliva)

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM480, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Stabilized Saliva DNA Kit (Cat.# AS1630)
- Optional: Proteinase K Solution (Cat.# MC5005)
- RNase A Solution (Cat. # A7973)
- Vortex mixer
- Heat block at 56°C
- GeneFix™ Saliva DNA Collection Device (Isohelix, Cat.# GFX-02)

Protocol:

1. Collect 2ml of saliva with the GeneFix™ Saliva DNA Collection Device per product instructions. Note: Storing the collectors containing saliva samples for more than two days after collection may improve extraction.
2. Prior to purification, vortex collection device for 10 seconds.
3. Transfer a 1ml aliquot of sample to a 1.5ml microtube.
4. (Optional) This step might increase DNA yields for samples that have been stored for less than two days before purification
    - Add 30µl Proteinase K solution.
    - Incubate samples at 56°C for 1 hour.
5. Transfer 1ml of sample to well #1 of the prepared Maxwell® RSC cartridge (see TM480 for instructions on how to prepare the cartridge).
6. Place one of the supplied elution tubes into the sample rack and add 150µl of Elution Buffer per sample.
7. Place the plunger in the indicated position of each cartridge.
8. Add 5µl of RNase A to well #3 of each cartridge.

9. Transfer Maxwell® RSC rack to the Maxwell® RSC instrument.

10. Select and run the Stabilized Saliva DNA method on the Maxwell® RSC instrument.

Results: Saliva samples were collected from three individuals into GeneFix™ Saliva DNA Collection Devices. Collection tubes were left at room temperature for three days. 1 hr before sample processing, the tubes were placed on a tube rotator to mix samples. Samples were processed per the above protocol (n=4 per individual) with the optional Proteinase K solution step. DNA concentrations were determined using the QuantiFluor® ONE dsDNA System (Cat.# E4871). Absorbance ratios (quality ratios) were determined using the NanoDrop spectrophotometer (Thermo Fisher Scientific). DNA amplifiability was assessed with amplification of a 75bp amplicon.

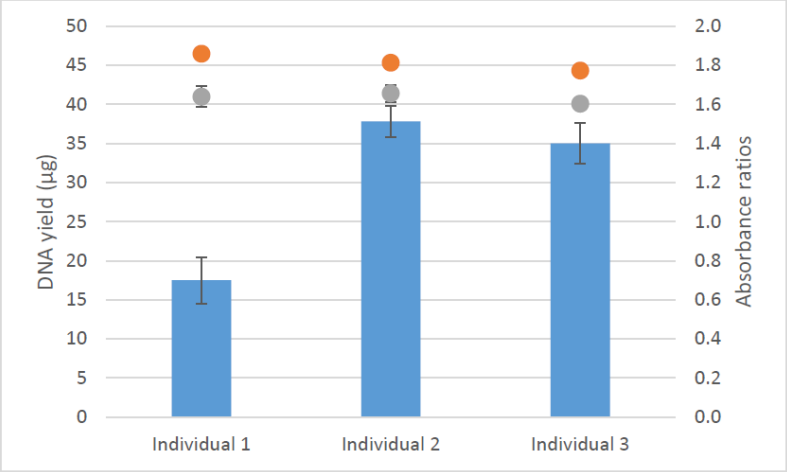

Figure 1. DNA yields and purity ratios from saliva samples determined by the QuantiFluor® ONE dsDNA System (Cat.E4871). The averaged DNA yields ranged from 17.5 to 37.8µg. The A260/A280 absorbance ratios ranged from 1.8 to 1.9 and the A260/A230 absorbance ratios ranged from 1.6 to 1.7.

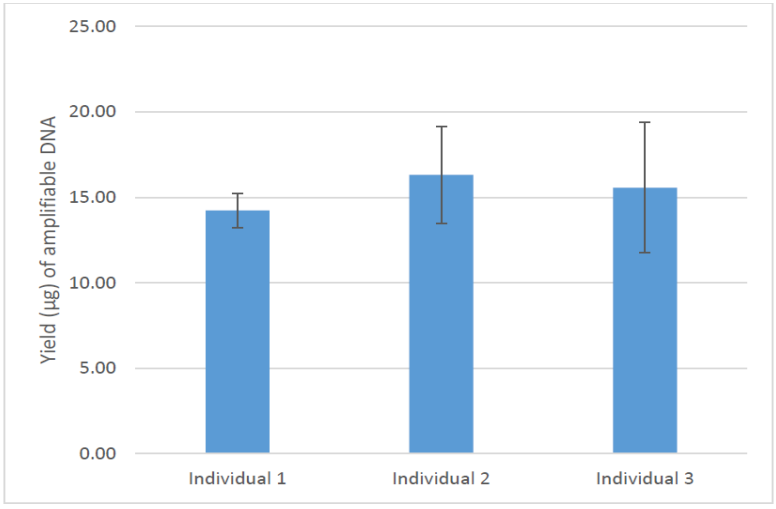

Figure 2. DNA yields from saliva samples determined by qPCR. The averaged DNA yields ranged from 14.2 to 16.3µg. No PCR inhibition was observed using an internal positive control.