Promega

Product Application

DNA Purification from Coffee Leaf using the Maxwell® RSC System

Isolate high-quality, amplifiable DNA from coffee leaf tissue using the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Plant DNA Kit (Cat.# AS1490)

Analyses: Quantification by fluorescent dye

qPCR amplification

Sample Type(s): Coffee Plant Leaf (*Coffea arabica)*

Input: 1–5 punches (~1.7mg/punch)

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 TM458, available at: www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Plant DNA Kit (Cat.# AS1490)
- 2.0ml screw-top tubes
- homogenization steel beads
- bead-beating device (MP Biomedicals Fast-Prep®-24 Instrument)
- microcentrifuge

Protocol:

1. Using a 4mm punch, place up to 5 punches into a 2ml screw-top tube with a steel bead.
2. Add 300µl of Tail Lysis Buffer and 10µl of RNase A solution to each sample tube.
3. Using a bead-beating device, homogenize samples for desired time (e.g., FastPrep®-24 Instrument at 4M/S, 20 seconds × 4, with 20-second delay).
4. Centrifuge the samples in a microcentrifuge at maximum speed for 2 minutes.
5. Add 300µl of Nuclease-Free Water to Well #1 of the Maxwell® cartridge.
6. Transfer the clarified supernatant to Well #1 of the Maxwell® cartridge.
7. Place elution tubes into the sample rack, and add 50µl of Elution Buffer for each sample.
8. Place the plunger in Well #8 of the Maxwell® cartridge.
9. Run the Maxwell® RSC Instrument with the Plant DNA protocol.

Results:

DNA was purified from coffee leaves with the Maxwell® RSC Plant DNA Kit using 1–5 punches input (approximately 1.7–8.7mg). High-molecular-weight, amplifiable DNA was obtained from all samples. Amplification inhibition in qPCR may occur with high sample input but can be alleviated by dilution of purified DNA (e.g., 1/10 dilution).

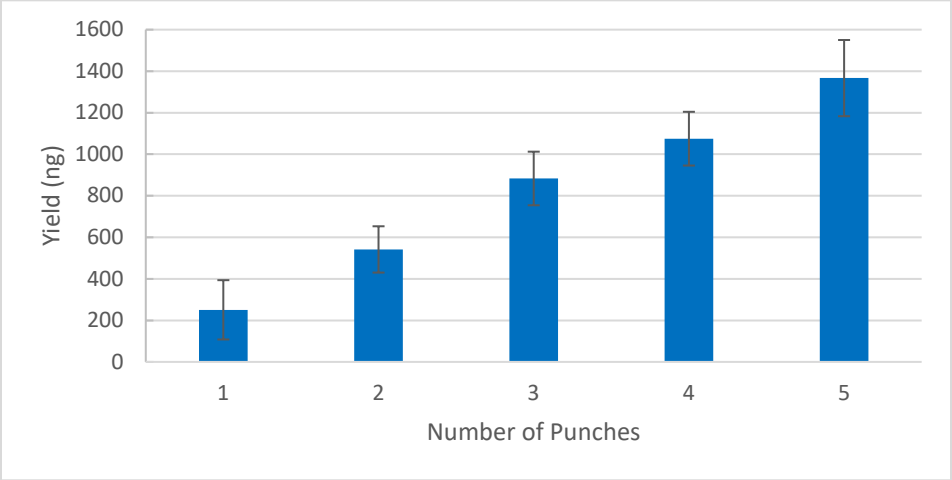

Figure 1. DNA yield obtained from coffee leaf using the Maxwell® RSC Plant DNA Kit. DNA was purified from 1–5 (4mm) punches and quantified using the QuantiFluor® ONE dsDNA System (Cat.# E4871) on a Quantus™ Fluorometer with a K562 Human Genomic DNA Standard. Mean ± STD shown (n=3).

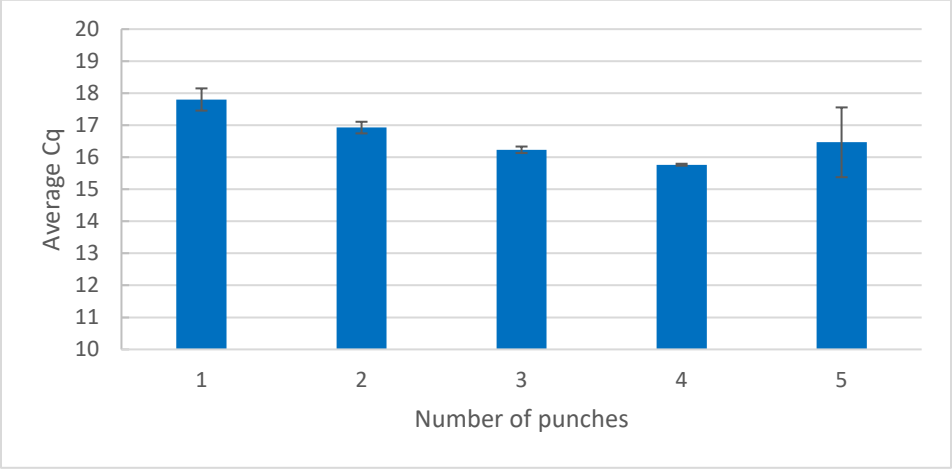

Figure 2. qPCR amplification of purified coffee leaf DNA. DNA eluates were amplified on a BioRad CFX96 using GoTaq® qPCR Master Mix (Cat.# A6001) with Universal Plant Primers (ATP-1; 1) using 2µl of undiluted purified DNA per 20µl reaction. Mean ± STD shown (n=3).

Reference:

1. Wang, J. *et al.* (2011) Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* **7**, 39.