Promega

Product Application

Coffee (*Coffea arabica*) Leaf DNA Purification

Isolate high quality, amplifiable DNA from coffee leaf tissue using the Maxwell® 16 System.

Kit: Maxwell® 16 LEV Plant DNA Kit (Cat. #AS1420)

Analyses: GoTaq® qPCR, QuantiFluor® quantitation, gel

Sample Type(s): *Coffea arabica* young plant leaves

Input: 1-5 punches of leaf tissue, 1 punch = ~ 2.5mg

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.

Further information can be found in Technical Manual #TM414, available at: **www.promega.com/protocols**

Materials Required:

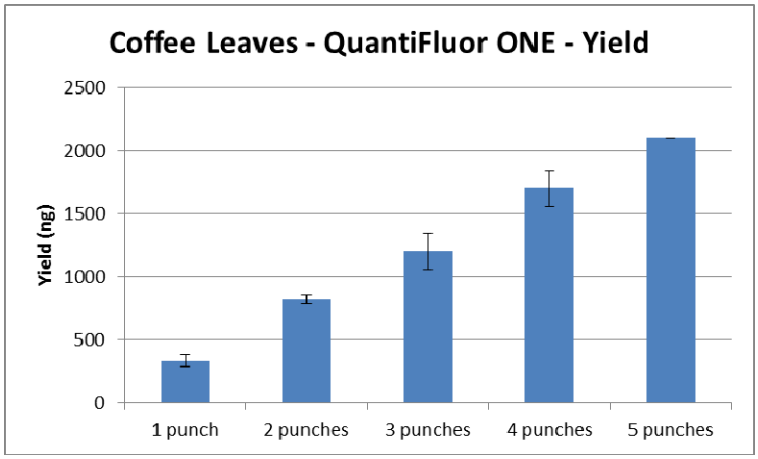
- Maxwell® 16 Instrument (Cat. #AS2000) with firmware version 4.97 or later
- Maxwell® 16 LEV Plant DNA Kit (Cat. #AS1420)
- 2.0mL screw-top tubes
- Homogenization steel bead
- Bead-beating device (e.g., MP Biomedicals FastPrep®-24 Instrument)
- Microcentrifuge

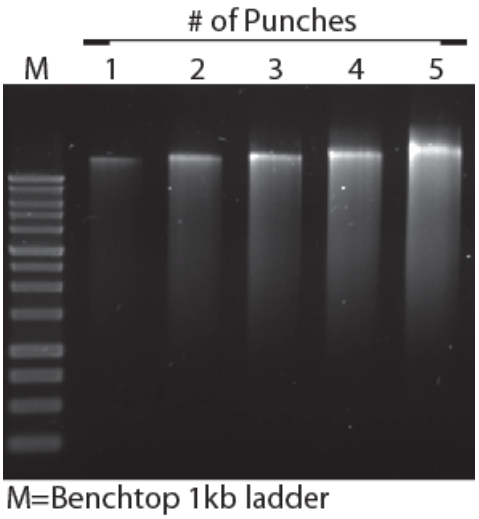
Protocol:

1. Using a 5mm punch, place desired number of punches (up to 5 punches) into a 2ml screw-top tube.
2. Add 300µl of Tissue Lysis Buffer (TLA) to each sample tube.
3. Using the bead-beating device, homogenize samples for desired time (e.g., FastPrep®-24 Instrument at 4M/S, 20seconds x 4 times with 20 second delay between each time).
4. Centrifuge samples in a microcentrifuge at max speed for 2 minutes.
5. Add 300µl of Nuclease Free Water to well #1 of each Maxwell cartridge.
6. Transfer the entire volume of supernatant to well #1 of the Maxwell cartridge.
7. Place one of the supplied elution tubes into the sample rack and add 50µl of the supplied Elution Buffer for each sample.
8. Place the plunger in the indicated position of the cartridge.
9. Select LEV configuration on the Maxwell® and select method: RUN, DNA: Plant. Start run.

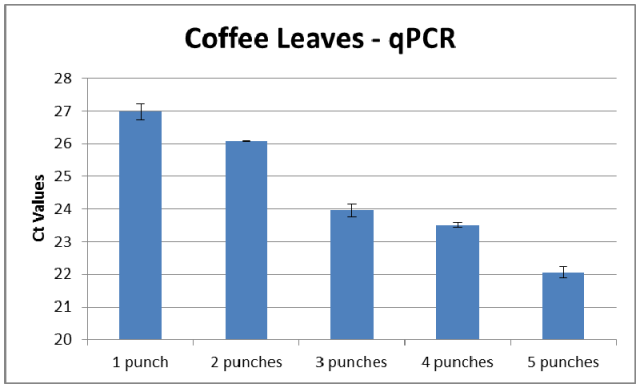
Results

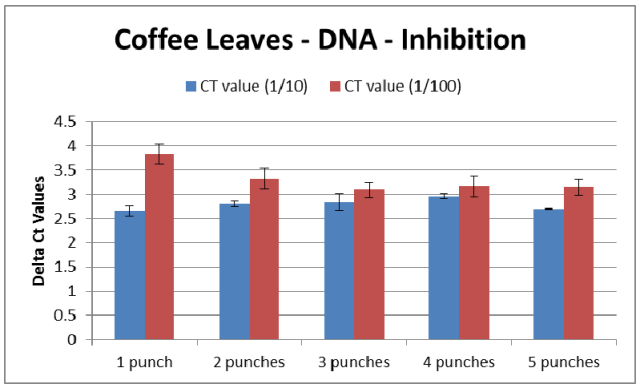
The above protocol was tested using 1-5, 5mm punches of Arabica coffee plant leaves.





Coffee leaf DNA yield and quality. **Left Panel:** DNA yield from 1-5 punches was quantified using the QuantiFluor® ONE dsDNA System (Cat. # E4871). **Right Panel:** Examples of high molecular weight DNA purified from the indicated number of punches and analyzed on a 1.0% Agarose gel with 5µl DNA eluate per lane. M = BenchTop 1kb DNA Ladder (Cat. # G7541).





Performance of purified DNA in qPCR. Left Panel: Analysis of purified DNA using GoTaq® qPCR Master Mix (Cat. # A6001) and Plant Universal Primers (1), using 1µl DNA eluate per 25µl reaction. **Right Panel:** ΔCt of serially diluted samples indicates minimal qPCR inhibition.

References:

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