Promega

Product Application

Coffee (*Coffea arabica*) Leaf RNA Purification

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

Kit: Maxwell® 16 LEV Plant RNA Kit (Cat. # AS1430)

Analyses: GoTaq® RT-qPCR, QuantiFluor® quantitation, gel

Sample Type(s): *Coffea arabica,* young plant leaf

Input: 1-5 punches 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 #TM415, available at: **www.promega.com/protocols**

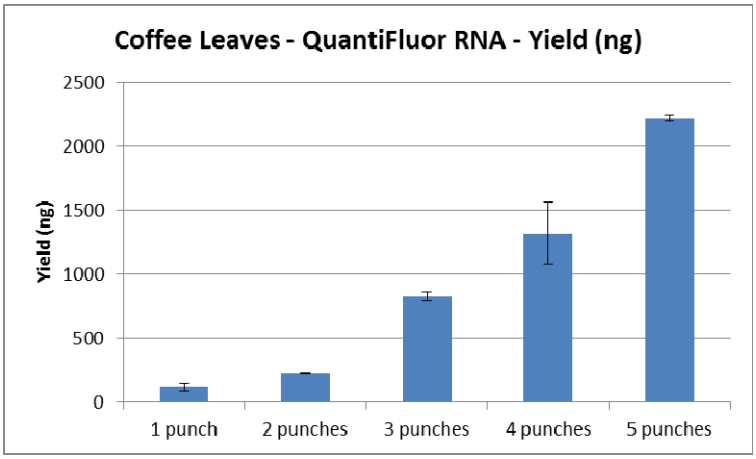
Materials Required:

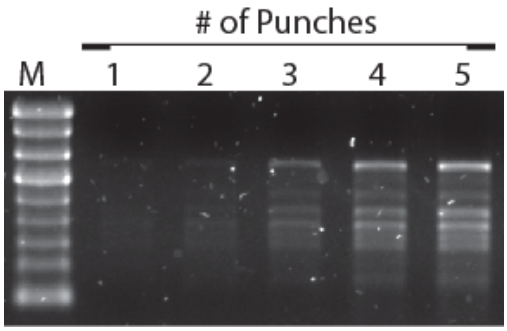
- Maxwell® 16 Instrument (Cat. #AS2000) with firmware version 4.97 or later
- Maxwell® 16 LEV Plant RNA Kit (Cat. #AS1430)
- 2ml screw-top tubes
- Bead-beating device (e.g., MP Biomedicals FastPrep®-24 Instrument) and homogenization steel bead
- Microcentrifuge

Protocol:

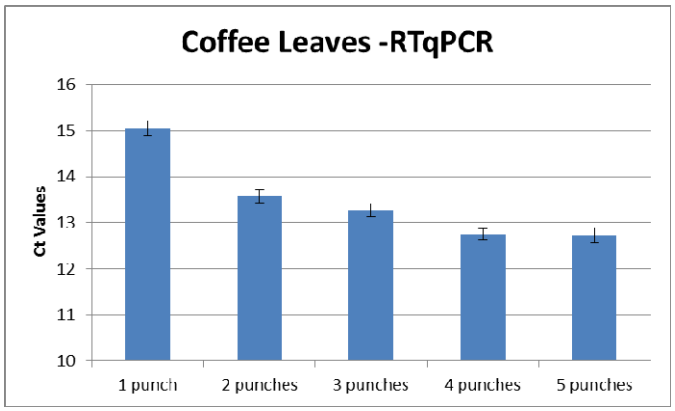
1. Using a 5mm punch, place desired number of punches (up to 5 punches) into a 2ml screw-top tube.
2. Add 400µl of chilled 1-Thioglycerol/Homogenization Solution to each sample tube.
3. Using the bead-beating device, homogenize sample for desired time (e.g., FastPrep®-24 Instrument at 4M/S, 20 seconds x 4 times with 20 second delay between each time).
4. Add 200µl of Lysis Buffer to the homogenate. Vortex vigorously for 15 seconds to mix.
5. Incubate samples at room temperature for 10 minutes.
6. Centrifuge samples in a microcentrifuge at max speed for 2 minutes.
7. Transfer the entire volume of supernatant to well #1 of the Maxwell® 16 LEV Cartridge.
8. Add 5µl of DNase to well #4.
9. Place one of the supplied elution tubes into the sample rack and add 50µl of the supplied Nuclease-Free Water for each sample.
10. Place the plunger in the indicated position of the cartridge.
11. Select LEV configuration on the Maxwell and select method: RUN, RNA: Plant. Start run.

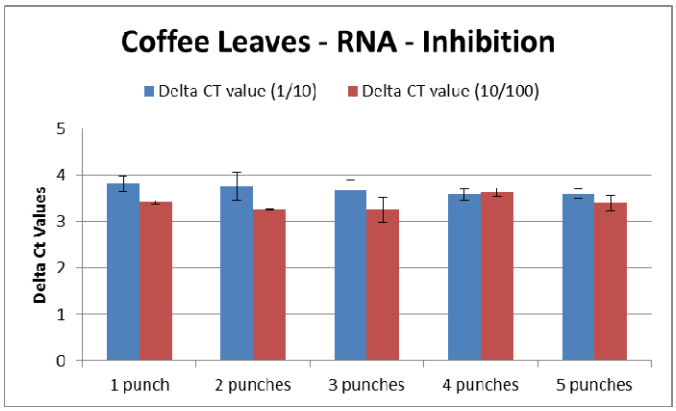
Results





RNA Yield and Quality. Left Panel: RNA yield from 1-5 leaf punches was quantified using the QuantiFluor® RNA System (Cat. # E3310). **Right Panel:** Examples of RNA purified from the indicated tissue masses analyzed on a 1.0% agarose gel with 5µl RNA eluate per lane. M=RNA Markers (Cat. # G3191).





Performance in RT-qPCR. Left Panel: Analysis of purified RNA using GoTaq® 1-Step RT-qPCR System (Cat. # A6020) with GAPDH primers (1) using 1µl RNA eluate per 20µl reaction. **Right Panel:** ΔCt of serially diluted RNA samples indicates minimal inhibition of RT-qPCR.

References:

1. Cruz, F., *et al.*, (2009). Evaluation of coffee reference genes for relative expression studies by quantitative real-time RT-PCR. Molecular Breeding. 23:4.