Promega

Product Application

DNA Isolation from Coffee Leaf Tissue using the ReliaPrep™ gDNA Tissue Miniprep System

Isolate high-quality, amplifiable DNA from coffee plant leaves using the ReliaPrep™ gDNA Tissue Miniprep System.

Kit: ReliaPrep™ gDNA Tissue Miniprep System (Cat.# A2051)

Sample Type: Coffee leaf tissue

Input: Up to 25mg

Materials Required:

- ReliaPrep™ gDNA Tissue Miniprep System (Cat.# A2051)
- 2.0ml screw-top tubes
- homogenization steel bead
- bead-beating device (e.g., MP Biomedicals FastPrep®-24 Instrument)
- microcentrifuge

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 TM345, available at: www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Protocol:

1. Using a 5mm punch, place desired number of punches (up to 25mg) into a 2ml screw-top tube.
2. To each sample add:
    - 100µl of Tail Lysis Buffer (TLA)
    - 300µl of Cell Lysis Buffer (CLD)
    - 20µl of RNase A Solution
    - 20µl of Proteinase K
3. Using the bead-beating device, homogenize samples for desired time (e.g., FastPrep®-24 Instrument at 4M/S, 20 seconds, 4 times with 20-second delay between each time).
4. Centrifuge samples in a microcentrifuge at max speed for 1 minute.
5. Incubate at room temperature for 10 minutes.
6. Centrifuge samples at max speed for 1 minute to reduce foaming.
7. Add 250µl of Binding Buffer (BBA) to each sample and vortex for 10 seconds.
8. Centrifuge samples at max speed for 2 minutes, and transfer liquid supernatant to a ReliaPrep™ Binding Column inside a collection tube.
9. Centrifuge samples at max speed for 1 minute. Transfer column to a new collection tube; discard the flowthrough and used collection tube.
10. Add 500µl of Column Wash Solution (CWD) to the sample and centrifuge at max speed for 2 minutes. Repeat this wash step for a total of 3 times, discarding liquid and collection tubes after every wash.
11. Eluates are ready for use in downstream applications.

Results:

Sample Type	NanoDrop		QuantiFluor® ONE	
	A_{260}/A_{280}	A_{260}/A_{230}	ng/µl	Yield (µg)
Coffee	2.19	2.03	13.67	0.62

Table 1. Coffee leaf DNA concentration, yield and purity based on quantitation using the QuantiFluor® ONE dsDNA System (Cat.# E4871) and the NanoDrop®-1000. DNA of high purity was recovered with purity ratios for samples >2.00. N=3.

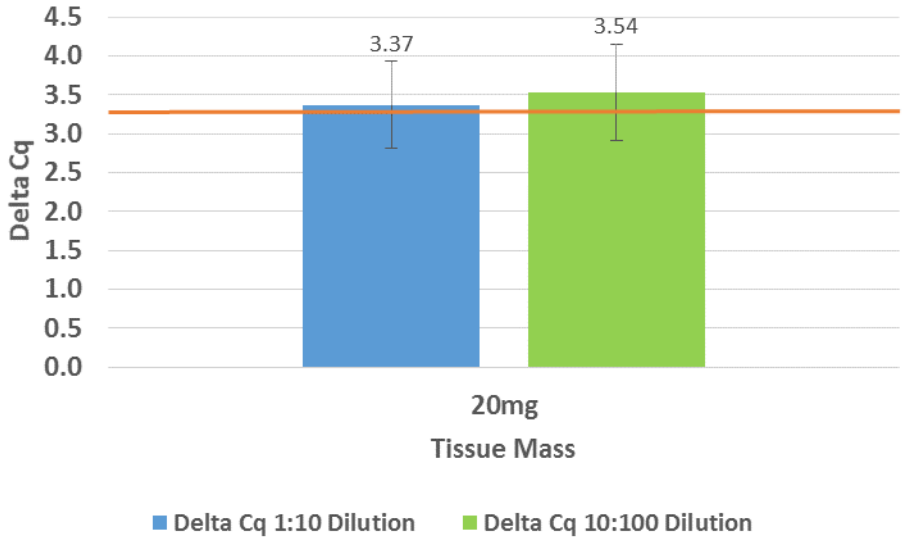

Figure 1. Inhibition analysis of purified coffee leaf DNA. DNA samples were serially diluted 1:10 and 10:100. For a sample diluted tenfold, ΔCq values are expected to be 3.3. ΔCq values significantly less than 3.3 may indicate the presence of inhibitors. ΔCq values of plant tissue samples indicate little to no inhibition of the serially diluted eluates. N=3.