Promega

Product Application

Isolating DNA from Cotton Leaf Tissue Using the ReliaPrep™ gDNA Tissue Miniprep System

Isolate high quality, amplifiable DNA from cotton leaf tissue using the ReliaPrep™ gDNA Tissue Miniprep System.

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

Sample Type: Cotton leaf tissue

Input: Up to 25mg

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

OR For further information, please contact **techserv@promega.com**

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

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 6m/s, 40 seconds).
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 flow through 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. Place the column in a clean 1.5ml microcentrifuge tube.

12. Add 50µl Nuclease-Free Water to the column. Centrifuge for 1 minute at max speed. Eluates are ready for use in downstream applications.

Results:

Table 1. Cotton leaf DNA concentrations, yields, 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 >1.7. N=3.

Sample	NanoDrop			QuantiFluor® ONE	
	ng/µl	A_{260}/A_{280}	A_{260}/A_{230}	ng/µl	Yield (µg)
Cotton Leaf	81.62	2.02	1.75	33.67	1.52

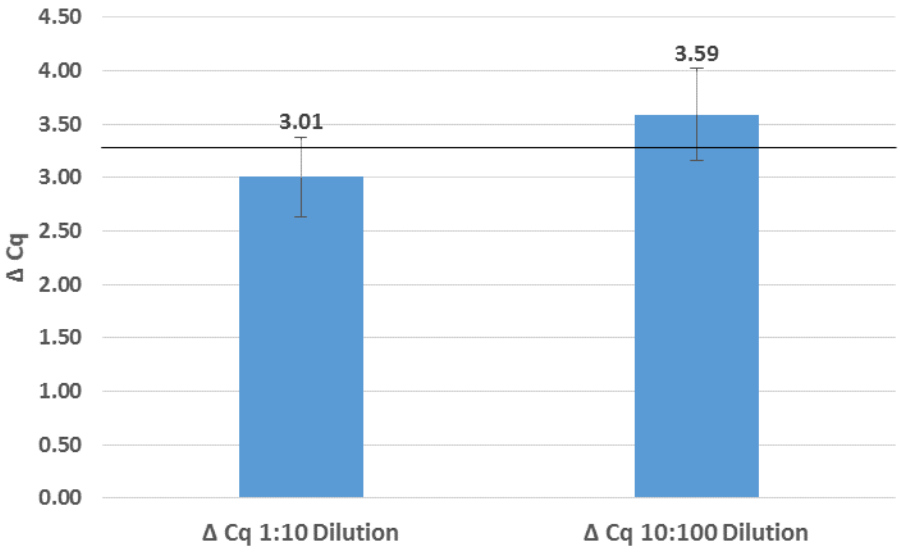

Figure 1. Inhibition analysis of purified cotton leaf DNA. DNA samples were serial diluted 1:10 and 10:100. For a sample diluted 10-fold, ΔCq values are expected to be 3.3. ΔCq values significantly less than 3.3 may indicate the presence of inhibitors. ΔCq values of DNA from cotton leaf samples indicate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

