

DNA Isolation from Corn and Soy Leaf Tissue Using the ReliaPrep™ gDNA Tissue Miniprep System

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

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

Sample Type(s): Corn and soy leaf tissue

Input: Up to 25mg

Materials Required:

- ReliaPrep™ gDNA Tissue Miniprep System (Cat.# A2051)
- liquid nitrogen
- mortar and pestle
- microtubes
- centrifuge

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. Grind leaves with a mortar and pestle using liquid nitrogen.
2. Measure up to 25mg of leaf tissue and add to microtube.
3. To each sample add:
 - a. 100µl of Tail Lysis Buffer (TLA)
 - b. 300µl of Cell Lysis Buffer (CLD)
 - c. 20µl of RNase A Solution
 - d. 20µl of Proteinase K
4. Vortex samples for 10 seconds.
5. Incubate at room temperature for 10 minutes.
6. Add 250µl of Binding Buffer (BBA) to each sample and vortex for 10 seconds.
7. Centrifuge samples at max speed for 2 minutes, and transfer liquid supernatant to a ReliaPrep™ Binding Column inside a collection tube.
8. Centrifuge samples at max speed for 1 minute. Transfer column to a new collection tube; discard the flow through and used collection tube.
9. 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.
10. Once washed, transfer the column to a clean 1.5ml tube. Add 50µl of Nuclease-Free Water to each sample and centrifuge at max speed for 1 minute. Discard the column.
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)
Corn	2.03	1.85	17.33	0.78
Soy	2.07	2.02	23.00	1.04

Table 1. Plant 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 >1.80. N=3.

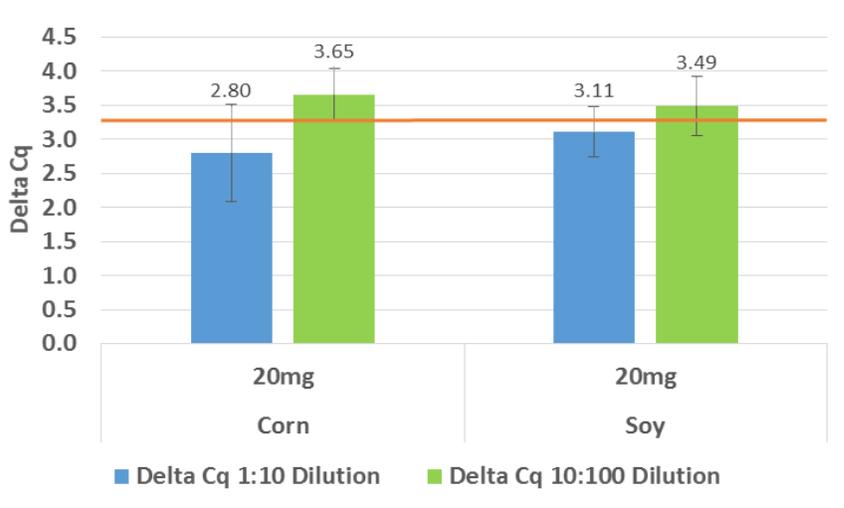


Figure 1. Inhibition analysis of purified plant leaf DNA. DNA samples were serially diluted 1:10 and 10:100. For a sample diluted tenfold, ΔCq values are expected to be 3.3. ΔCq values significantly less than 3.3 may indicate the presence of inhibitors. ΔCq values of plant tissue samples indicate little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