Promega

Product Application

DNA Purification from Cotton Leaf using the Maxwell® RSC System

Isolate high-quality, amplifiable DNA from cotton leaf tissue using the Maxwell® RSC System.

Kit: Maxwell® RSC Plant DNA Kit (Cat.# AS1490)

Analyses: NanoDrop, QuantiFluor® quantitation, agarose gel electrophoresis, qPCR

Sample Type(s): Cotton, *Gossypium herbacium*

Input: 1–5 punches (~2.1mg/punch)

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 TM458, available at: www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Plant DNA Kit (Cat.# AS1490)
- 2.0ml screw-top tubes
- homogenization Beads
- bead-beating device (MP Biomedicals Fast-Prep®-24 Instrument)
- microcentrifuge

Protocol:

1. Using a 4mm punch, place up to 5 punches into a 2ml screw-top tube.
2. Add 300µl of Tissue Lysis Buffer and 10µl of RNase A solution to each sample tube.
3. Using the bead-beating device, homogenize samples for desired time (e.g., FastPrep®-Instrument at 4M/S, 20 seconds, 4 times with 20-second delay between each time).
4. Centrifuge the samples in a microcentrifuge at maximum speed for 2 minutes.
5. Add 300µl of Nuclease-Free Water to Well #1 of the 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 Well #8 of the cartridge.
9. On the Maxwell® RSC, select the Plant DNA Kit protocol. Start Run.

Results:

DNA was purified from cotton leaves with the Maxwell® RSC Plant Kit using 1–5 punches of input (approximately 0.0021–0.0105g). DNA dilution may be required for uninhibited amplification with higher sample input amounts.

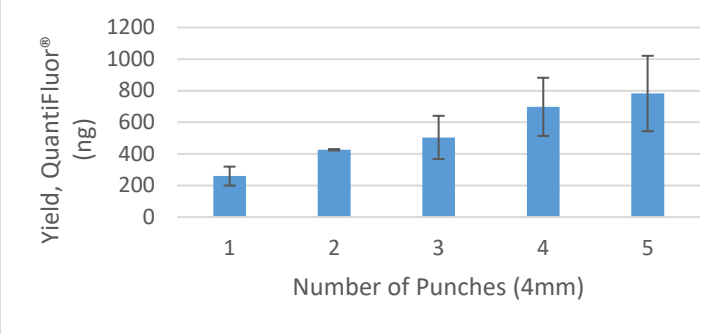

Figure 1. DNA yield purified from cotton leaf and measured by QuantiFluor® ONE dsDNA System (Cat.# E4871).

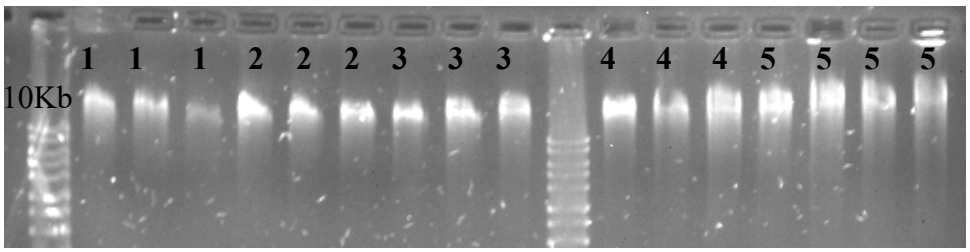

Figure 2. DNA integrity for purified cotton DNA. Examples of high-molecular-weight DNA purified from the indicated number of punches and separated by electrophoresis on a 1% agarose gel.

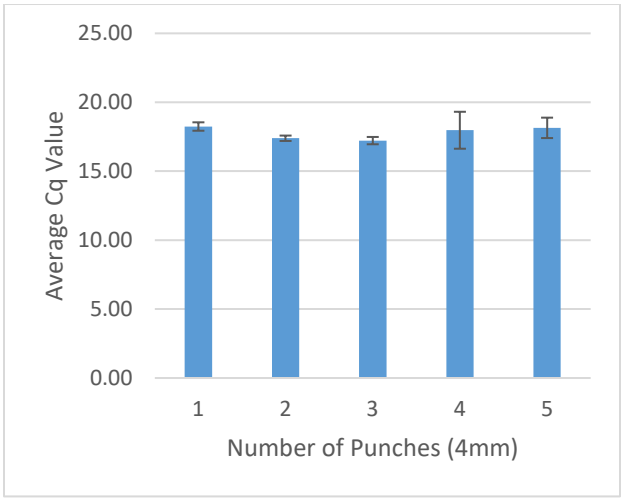

Figure 3. qPCR amplification of purified cotton DNA. GoTaq® qPCR amplification with Universal Plant Primers (ATP-1) using 2µl of DNA eluate per reaction.