Promega

Product Application

RNA Purification from Plant Leaves using the Maxwell® RSC System

Isolate high-quality, amplifiable RNA from various plant leaves using the Maxwell® RSC System.

Kit: Maxwell® RSC Plant RNA Kit (Cat.# AS1500)

Analyses: NanoDrop, QuantiFluor® quantitation, TapeStation

Sample Type(s): *Z. mays* (corn), *G. max* (soybean), *C. arabica* (coffee), *G. herbaceum* (cotton), *C. annuum* (jalapeño), *E. guineensis* (oil palm), *L. lamium* (cucumber), *S. lycopersicum* (tomato), *C. sativus* (mint), *H. echinacea* (cone flower)

Input: 100mg of leaf tissue

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 TM459, available at: www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Plant RNA Kit (Cat.# AS1500)
- microcentrifuge
- bead beating device (e.g., FastPrep 24, MP Biomedicals)
- bead beating tubes (e.g., 2ml screw cap tubes)
- steel beads (e.g., Spex 2150)

Protocol:

1. Weigh 100mg of plant leaf material, and place it in a 2ml screw cap tube with 1 steel bead.
2. Add 400µl of chilled 1-Thioglycerol/Homogenization solution. If there is plant material on the cap or sides of the tube above the homogenization solution, pulse-spin the sample at maximum speed in the tube.
3. Add 200µl of Lysis Buffer and vortex for 15 seconds to mix.
4. Place the tubes in the FastPrep™-24 Bead Beater and process at 4M/S, 20 seconds × 4, with a 20-second delay between each cycle. Repeat for a total of 2 runs.
5. Centrifuge the sample at maximum speed in a microcentrifuge for 2 minutes.
6. Prepare the Maxwell® RSC Cartridge.
   - Place plungers in well #8.
   - Place 0.5ml elution tubes in the front of the deck tray, and add 50µl of Nuclease-Free Water.
   - Add 5µl of DNase into Well #4. The solution should turn green.
7. Transfer the clear supernatant to Well #1 of the Maxwell® RSC Cartridge.
8. Place in the Maxwell® RSC Instrument, and run the Plant RNA protocol.

Results:

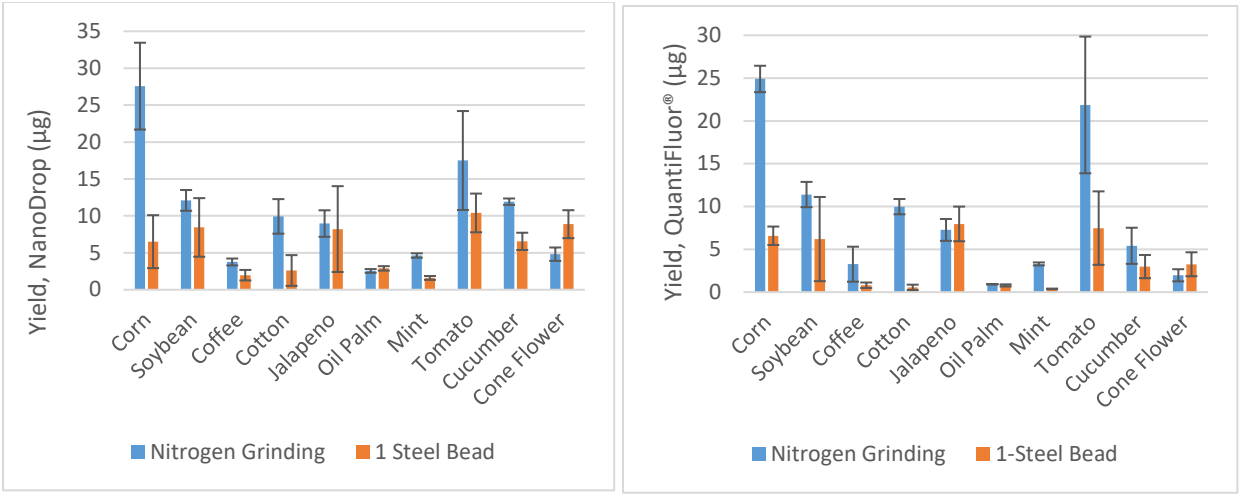

Figure 1. RNA yield from various leaf sources using the Maxwell® RSC Plant RNA Kit. RNA was purified from 100mg of leaf tissue after grinding in liquid nitrogen or bead beating with a single steel bead. Yield was measured by absorbance (left) and fluorescence (QuantiFluor® RNA System; right). Mean ± SD shown (n=3).

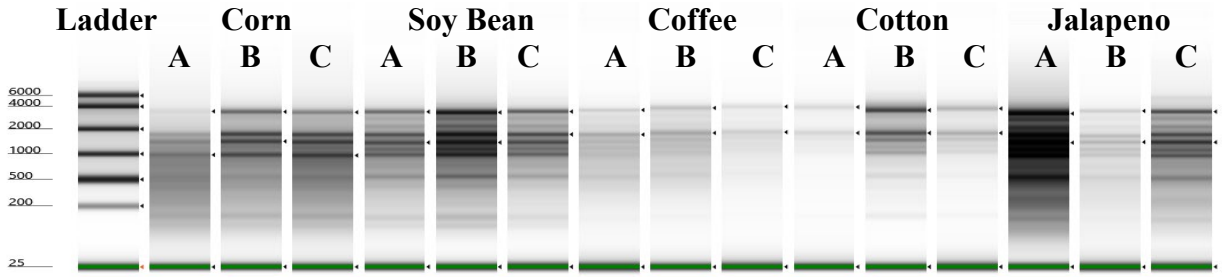

Figure 2. RNA integrity and quantity for various leaves using beat beating and the Maxwell® RSC Plant RNA Kit. RNA was purified from 100mg of plant leaf after grinding in liquid nitrogen. Integrity was tested using RNA ScreenTape on an Agilent 4200 TapeStation according to the technical manual.

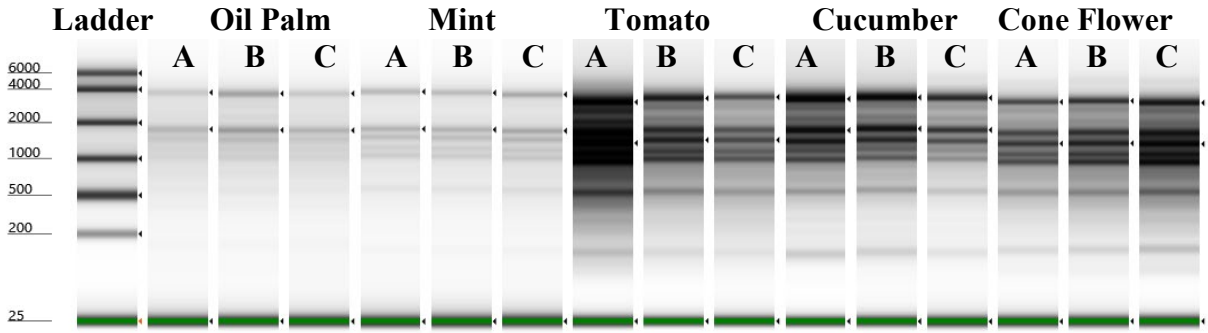

Figure 3. RNA integrity and quantity for various leaves using bead beating and the Maxwell® RSC Plant RNA Kit. RNA was purified from 100mg of plant leaf input using bead beating with one steel bead. Integrity was tested using RNA ScreenTape on an Agilent 4200 TapeStation according to the technical manual.