Promega

Product Application

Automated Purification of RNA from Strawberry Leaves

Purify RNA from strawberry leaves using the Maxwell® RSC Instrument and the Maxwell® RSC Plant RNA Kit.

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

Analyses: Dye-based quantitation and RT-qPCR

Sample Type(s): Strawberry *(Fragaria x ananassa)* leaves

Input: 20-50mg of strawberry leaf

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 Plant RNA Kit (Cat.# AS1500)
- CTAB buffer (Cat.# MC1411)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Microcentrifuge
- Heat block capable of 65°C
- FastPrep®Tubes 2ml (MP Biomedicals, Cat.# 5076-400)
- SPEX Sample prep steel beads (Cat.# 2150)
- Bead-Beating Device (BioSpecs Products)

Protocol:

1. Weigh out 20mg or 50mg of strawberry leaves from 6mm punches and place into a 2ml screw-top tube.
2. Add 600µl of CTAB buffer supplemented with 2% 1-thioglycerol and homogenize samples using a bead-beating device.
3. Place samples in a standard heat block at 65°C for 15 minutes.
4. Centrifuge for 5 minutes at 15,000 *x g* to separate any solids.
5. Transfer 400µl of the cleared sample supernatant to a clean 1.5 microcentrifuge tube.
6. Add 200µl of Lysis Buffer to the cleared supernatant and mix by vortexing at maximum speed for 10 seconds.
7. Incubate at room temperature for 10 minutes and transfer the lysate into well #1 of the Maxwell® RSC cartridge.
8. Add 5µl of DNase I solution to well #4. Add plungers to well #8 of the Maxwell® RSC cartridge.
9. Place the supplied elution tubes into the sample rack and add 50µl of the Nuclease-Free Water.
10. Run the method *Maxwell® RSC Plant RNA Kit* on the Maxwell® RSC Instrument.

Results:

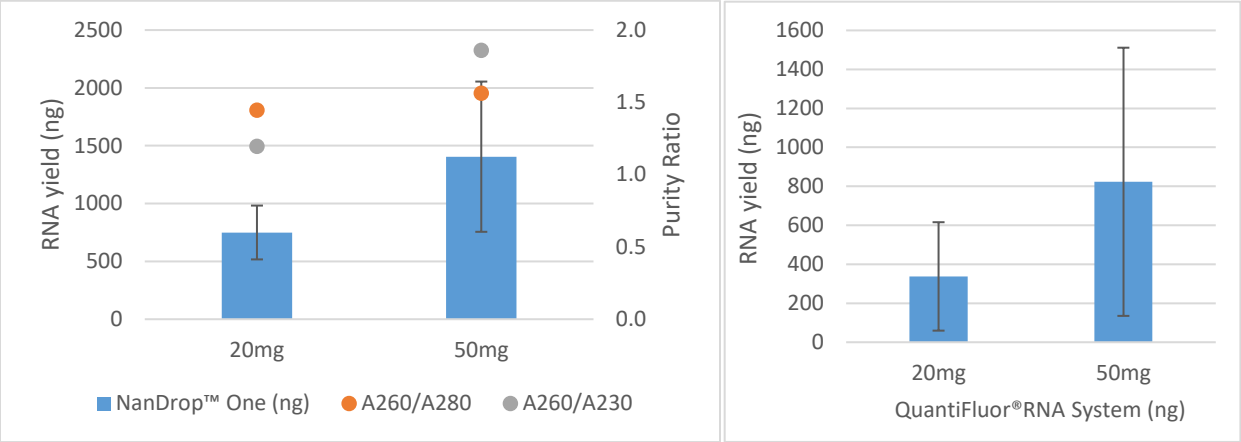

Figure 1. Quantification of RNA isolated from strawberry leaves. RNA was isolated from 20mg or 50mg of strawberry leaves using the Maxwell® RSC Plant RNA Kit (Cat.# AS1500) modified with CTAB buffer (Cat.# MC1411) as the homogenization buffer. The RNA yield and purity ratio were quantified using both **(Left)** NanoDrop™ One and **(Right)** QuantiFluor® RNA System (Cat.# E3310). Average values ± STD from three experiments are shown.

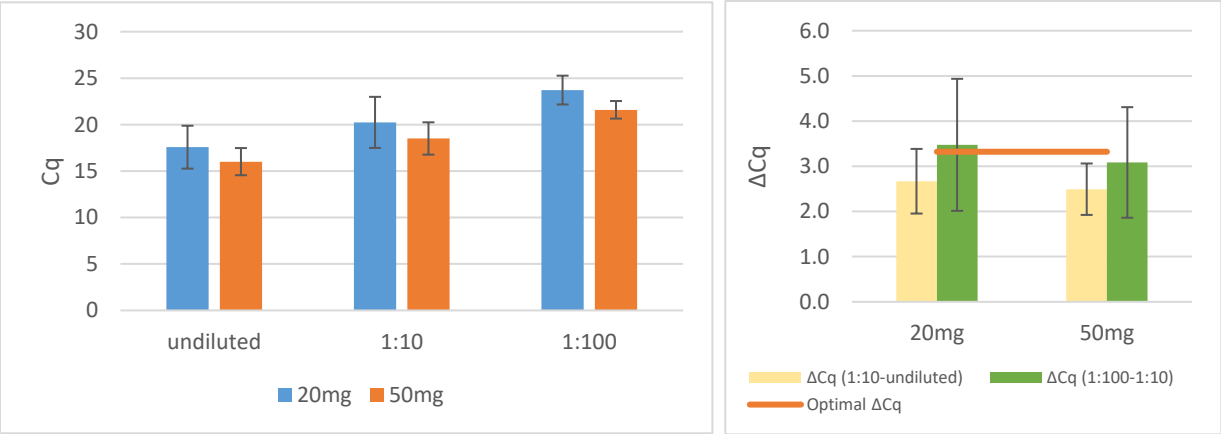

Figure 2. qPCR amplification of RNA isolated from strawberry leaf. Strawberry RNA was detected using GoTaq® 1-Step RT-qPCR System (Cat.# A6020). Cq value of different amount of input materials was shown. Undiluted RNA eluate prepared from both 20mg and 50mg of strawberry leaves was diluted at 1:10 and 1:100 and amplified with strawberry specific primers[1] to examine the amplification efficiency. No significant inhibitors were found to be co-purified with the RNA according to the ΔCq analysis. Average values from three experiments ± STD are shown.

References:

1. Amil-Rutz et al (2013) Identification and Validation of Reference Genes for Transcript Normalization in Strawberry *(Fragaria x ananassa)* Defense Responses. PLoS ONE (8): e70603.