Promega

Product Application

Berry Plant Leaf RNA Purification

Isolate high quality, amplifiable RNA from leaves of berry plants using the Maxwell® 16 System.

Kit: Maxwell® 16 LEV Plant RNA Kit (Cat. # AS1430)

Analyses: GoTaq® RT-qPCR, QuantiFluor® quantitation

Sample Type(s): Strawberry (*Fragaria ananassa*)
Raspberry (*Rubus sp.*)
Gooseberry (*Ribes uva-crispa*)

Input: Up to 50mg 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.

Further information can be found in Technical Manual #TM415, available at: **www.promega.com/protocols**

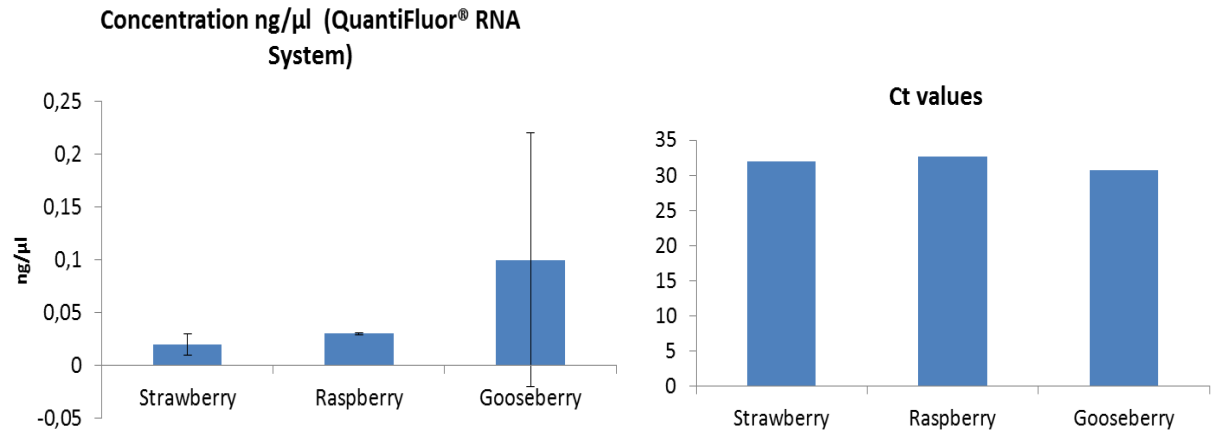
Materials Required:

- Maxwell® 16 Instrument (Cat. #AS2000) with firmware version 4.97 or later
- Maxwell® 16 LEV Plant RNA Kit (Cat.#AS1430)
- Bead-beating device (e.g., MP Bio FastPrep®-24 Instrument and D Lysing Matrix tubes, Cat. #6913-100)
- Microcentrifuge

Protocol:

1. Cut and weigh 50 mg of leaf tissue.
2. Place the leaf tissue into the lysing matrix tube.
3. Add 600 µl of 1-Thioglycerol/Homogenization Solution to each sample.
4. Run the bead-beating device using the time and speed recommended by the manufacturer. (e.g., FastPrep®-24 Instrument for 80 seconds at 6 m/s.)
5. Transfer 400 µl of homogenate to a new tube and add 200 µl of Lysis Buffer. Vortex vigorously for 15 seconds to mix.
6. Incubate at room temperature for 10 minutes. Spin the sample at maximum speed in a microcentrifuge for 2 minutes.
7. Transfer the supernatant to well #1 of the Maxwell® 16 LEV Cartridge.
8. Add 5 µl of DNase to well #4.
9. Place one of the supplied elution tubes into the sample rack and add 50µl of the supplied Nuclease-Free Water for each sample.
10. Place the plunger in the indicated position of the cartridge.
11. Select LEV configuration on the Maxwell® Instrument and select the purification method as follows: RUN, RNA: Plant. Start run.

Results



Concentration and performance of extracted RNA. Left Panel: RNA concentratation was determined by quantitation using the QuantiFluor® RNA System (Cat.# E3310). **Right Panel:** C_t values were determined using the GoTaq®1-Step RT-qPCR System (Cat.# A6020), universal plant primers and 1 µl RNA eluate per 50µl reaction.