

Deciduous Fruit Tree Leaf RNA Purification

Isolate high quality, amplifiable RNA from leaves of deciduous fruit trees using the Maxwell® 16 System.

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

Analyses: GoTaq® RT-qPCR, QuantiFluor® quantitation

Sample Type(s): Peach (*Prunus persica*) leaf
Apple (*Malus domestica*) leaf
Fig tree (*Ficus carica*) leaf
Persimmon (*Diospyros kaki*) leaf
Plum (*Prunus sp.*)
Apricot (*Prunus armeniaca*)
Cherry (*Prunus avium*)
Quince (*Cydonia oblonga*)
Medlar of Holland (*Eriobotrya germanica*)

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

Input: up to 50mg leaf tissue

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

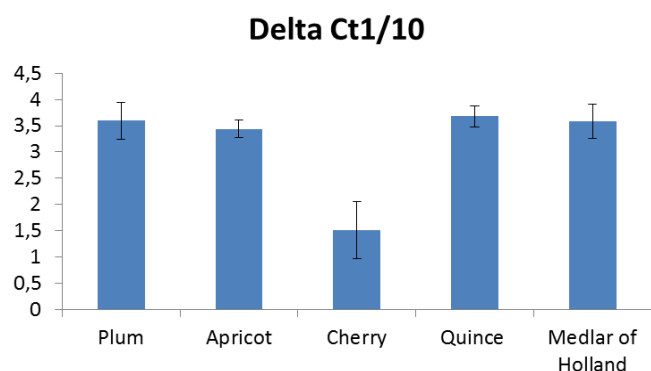
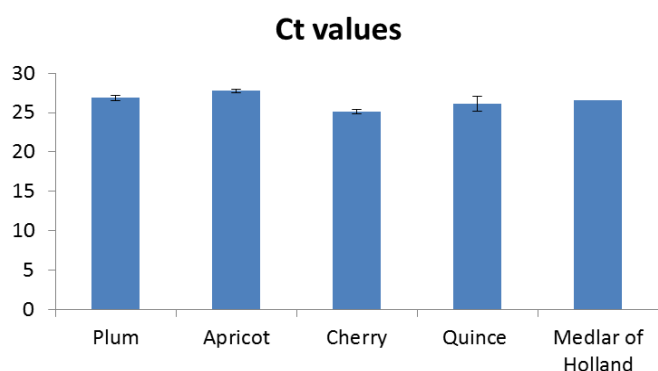
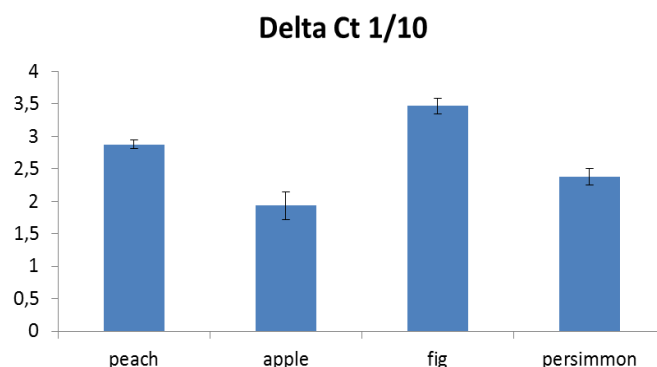
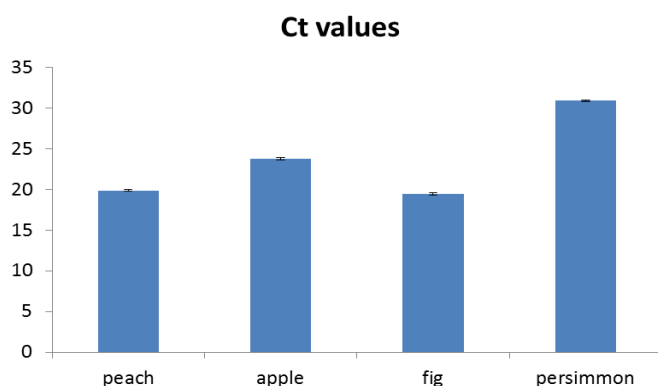
Protocol:

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

Results:

Plant	Concentration (ng/μl)	SD
Peach	150.3	34.59
Apple	1.7	1.3
Fig	132.9	64
Persimmon	0.01	0.02
Plum	0.8	0.23
Apricot	0.3	0.12
Cherry	1.4	0.51
Quince	1.1	0.19
Medlar of Holland	0.5	0.08

Concentration of purified RNA. RNA was purified from leaf tissue of the indicated fruit trees as described in the above protocol. RNA was eluted in 50μl and the concentration calculated using the QuantiFluor® RNA System (Cat. #E3310).



Performance in RT-qPCR. **Left Panels:** Ct values determined using GoTaq®1-Step RT-qPCR System (Cat. # A6020), universal plant primers, and 1μl RNA eluate per 50μl reaction. **Right Panels:** Changes in Ct values of serially diluted samples.