Promega

Product Application

Deciduous Fruit Tree Leaf DNA Purification

Isolate high quality, amplifiable DNA from deciduous fruit tree leaves using the Maxwell® 16 System.

Kit: Maxwell® 16 LEV Plant DNA Kit (Cat.# AS1420)

Analyses: GoTaq® qPCR, QuantiFluor® quantitation

Sample Type(s): Peach (*Prunus persica*) leaf
Apple (*Malus domestica*) leaf
Fig tree (*Ficus carica*) leaf
Persimmon (*Diospyros kaki*) leaf

Input: up to 20mg 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 #TM414, available at: **www.promega.com/protocols**

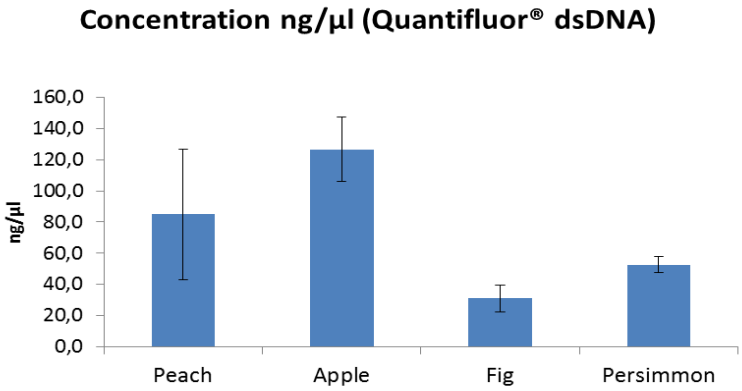
Materials Required:

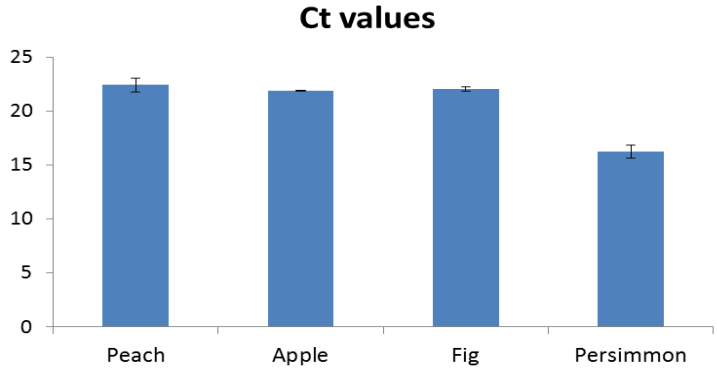
- Maxwell® 16 Instrument (Cat. #AS2000) with firmware version 4.97 or later
- Maxwell® 16 LEV Plant DNA Kit (Cat.#AS1420)
- Bead-beating device (e.g., MP Bio FastPrep®-24 Instrument)
- D lysing Matrix tubes for use with MP Bio FastPrep®-24 Instrument (Ref. 6913-100)
- Microcentrifuge

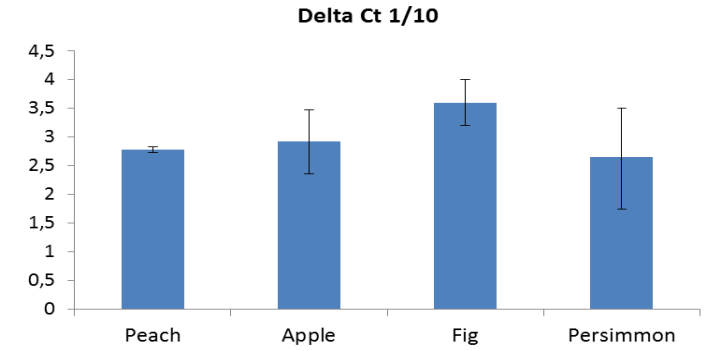
Protocol:

1. Cut and weigh 20mg leaf tissue.
2. Place the leaf tissue into the lysing matrix tube.
3. Add 300µl of Tail Lysis Buffer (TLA) to each sample.
4. Add 10µl of RNase A (optional) to each sample.
5. Run the bead-beating device using the time and speed recommended by the manufacturer.
6. Place the extraction tubes into a centrifuge and spin briefly to remove any solid particulates.
7. Add 300µl of Nuclease Free Water to well #1 of each Maxwell® Reagent Cartridge. Transfer all liquid and any remaining foam to well #1, being careful not to transfer any solid material to the cartridge.
8. Place one of the supplied elution tubes into the sample rack and add 50µl of the supplied Elution Buffer 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, DNA: Plant. Start run.

Results







Concentration and quality of extracted DNA. Top Panel: DNA was extracted from 20mg leaf tissue and eluted in 50µl. DNA concentration was calculated using the QuantiFluor® dsDNA System (Cat.# E2670). Values represent the mean and standard deviation of n=3 samples of each type. **Middle Panel:** Ct values were determined using GoTaq® qPCR Master Mix (Cat.# A6001), using universal plant primers and 1 µl DNA eluate in a 50 µl reaction. **Bottom Panel:** Changes in the Ct values of serially diluted samples indicate minimal inhibition of qPCR.