Promega

Product Application

“Vegetables” leaves RNA Purification

Isolate high quality, amplifiable RNA from vegetables leaves tissue using the ReliaPrep™ RNA miniprep system.

Kit: ReliaPrep™ RNA Tissue Miniprep System (Cat. #Z6111)

Analyses: GoTaq® RT-qPCR, QuantiFluor® quantitation

Sample Type(s): Tomato (*Solanum lycopersicum*) leaf
Potato (*Solanum tuberosum*) leaf
Eggplant (*Solanum melongena*) leaf*
Zucchini (*Cucurbita pepo*) leaf

*Eggplant was collected in spring and autumn. The rest of leaves were collected only in spring.

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 #TM394, available at: **www.promega.com/protocols**

Input: up to 50mg leaf tissue

Materials Required:

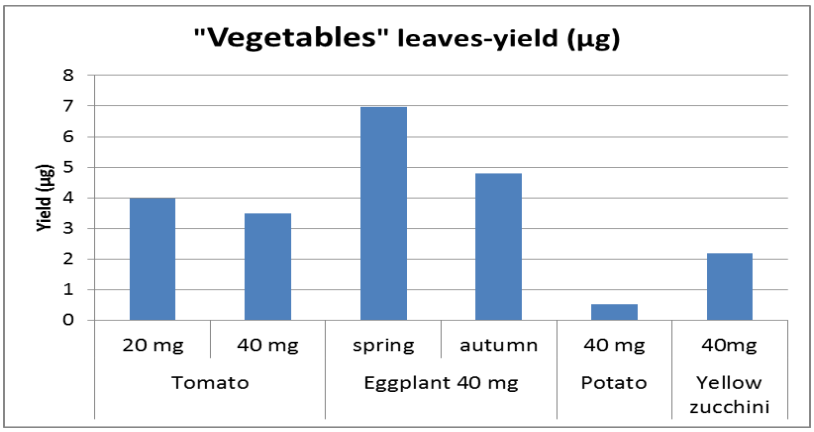
- ReliaPrep™ RNA Tissue Miniprep System (Cat. #Z6111)
- Blade
- D lysing matrix (ref 6913-100)
- Isopropanol
- 95% Ethanol
- Microcentrifuge
- FastPrep®-24 Instrument

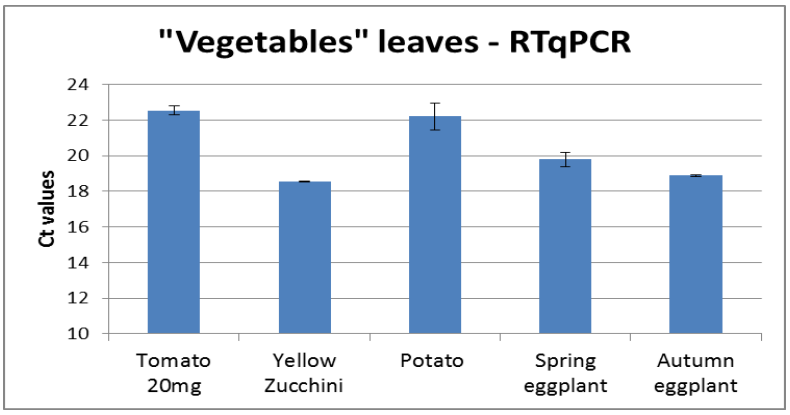
Protocol: (following the non-fibrous tissue protocol)

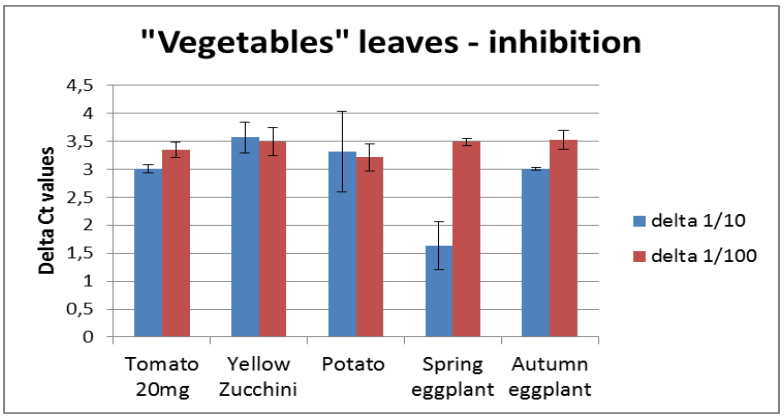
1. Prepare solutions as described in the technical manual (TM394).
2. Cut the leaf with the blade and weigh it.
3. Add up to 50mg of ground sample to a lysing matrix tube with the beads.
4. Add 500µl of LBA + TG Buffer to the tube.
5. Homogenize samples with FastPrep®-24 during 40 seconds at 6 m/s. Put the samples on ice 1 minute. Repeat the homogenization.
6. Clear homogenates by centrifugation for 3 minutes at 14,000 x *g*. (Optional: take all the homogenates to a clear tube to clear more efficiently without the beads)
7. Add 170µl of isopropanol. Mix by vortexing for 5 seconds.
8. Transfer lysate into a Reliaprep Minicolumn and centrifuge at 12,000-14,000 x *g* for 1 minute.

Proceed with the protocol in the technical manual (TM394) to purify the RNA using the ReliaPrep™ minicolumn.

Results:







Upper: Vegetables RNA yields are based on quantitation using the QuantiFluor™ RNA System (Cat. #E3310). **Medium:** Analysis of purified RNA using GoTaq® 1-Step RT-qPCR System (Cat. #A6020) with Universal plant primers (Reference 1) using 1µl RNA eluate per 50µl reaction. **Lower:** ΔCq of serial diluted samples indicate no inhibition of RT-qPCR.

Reference:

1. Wang et al.: Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. Plant Methods 2011 7:39.