Promega

Product Application

Coffee (*Coffea arabica)* Leaf and Coffee Bean RNA Purification

Isolate high quality, amplifiable RNA from coffee leaf tissue using the ReliaPrep™ RNA miniprep system.

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

Analyses: GoTaq® RT-qPCR, QuantiFluor® quantitation, gel

Sample Type(s): *Coffea arabica*

Input: up to 50mg leaf tissue or
20mg coffee bean (un-roasted)

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**

Materials Required:

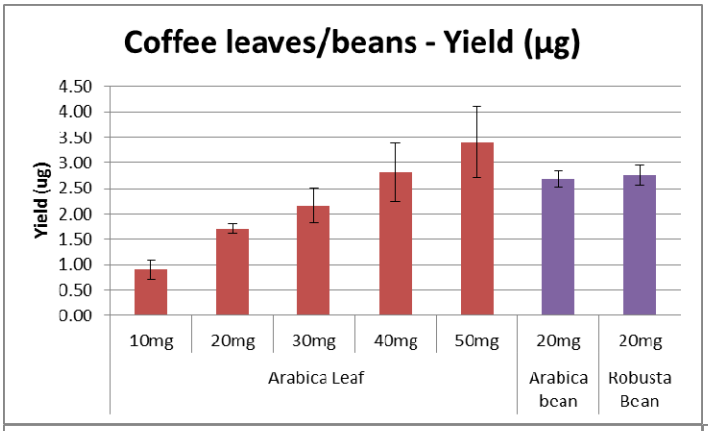
- ReliaPrep™ RNA Tissue Miniprep System (Cat. #Z6111)
- Liquid Nitrogen
- Mortar and Pestle
- Isopropanol
- 95% Ethanol
- Tissue Homogenizer (e.g., Tissue-Tearor™ homogenizer)
- Microcentrifuge

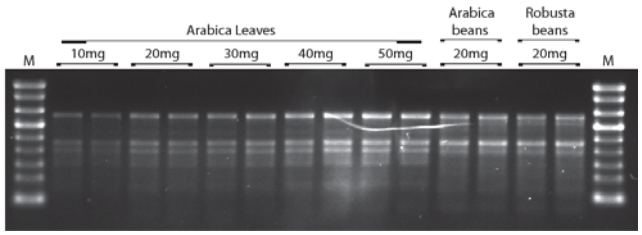
Protocol: (following the non-fibrous tissue protocol)

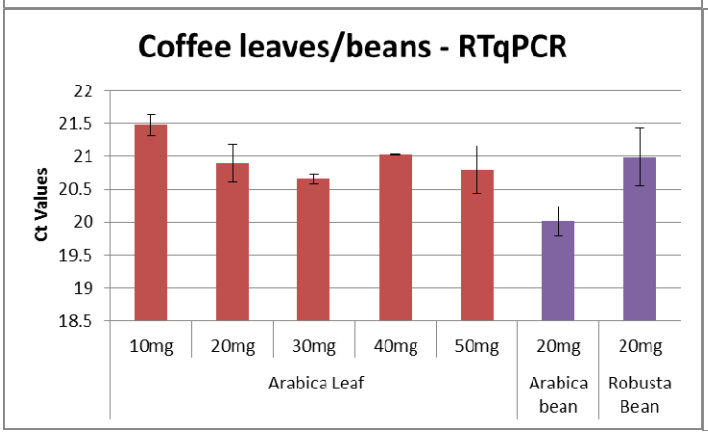
1. Prepare solutions as described in the technical manual (TM394).
2. Grind tissue or bean sample material in liquid nitrogen using a mortar and pestle.
3. Add up to 50mg of ground sample to a 2ml tube.
4. Add 500µl of LBA + TG Buffer to the tube.
5. Homogenize samples with a small tissue homogenizer for 30-60 seconds.
6. Clear homogenates by centrifugation for 3 minutes at 14,000 x *g*.
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.

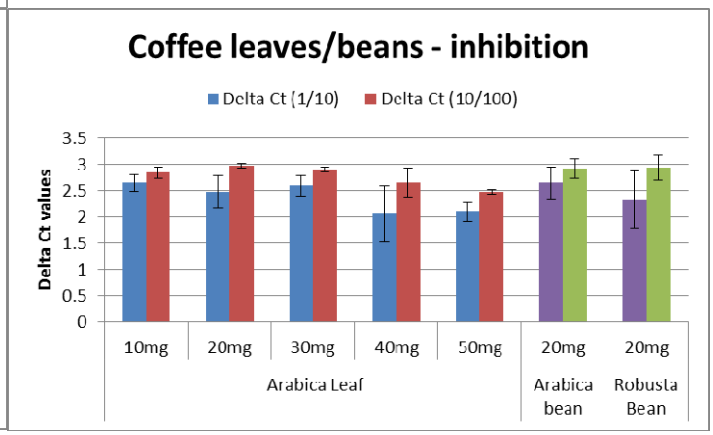
Proceed with the protocol in the technical manual (TM394) to purify the RNA using the ReliaPrep™ minicolumn.

Results:
The above protocol was tested using Arabica coffee leaves and Arabica and Robusta coffee beans.









Upper Left: Coffee leaf and bean RNA yields are based on quantitation using the QuantiFluor™ RNA System (Cat. #E3310). **Upper Right:** Examples of RNA purified from the indicated tissue masses analyzed on a 1.0% agarose gel with 5µl RNA eluate per lane. M=RNA Markers (Cat. #G3191) **Lower Left:** Analysis of purified RNA using GoTaq® 1-Step RT-qPCR System (Cat. #A6020) with GAPDH primers (Reference 1) using 1µl RNA eluate per 20µl reaction. **Lower Right:** ΔCq of serial diluted samples indicate no inhibition of RT-qPCR.

Reference:

1. Cruz, F., *et al.*, (2009). Evaluation of coffee reference genes for relative expression studies by quantitative real-time RT-PCR. Molecular Breeding. 23:4.