

Nucleic Acid Purification From Plant Tissues for Viral Detection

Samantha Lewis¹, Laurence Delaurière², Becky Carroll¹, Elisa Ruiz², Elyn Lepinski¹, Giuseppe Durante³, Caroline Chatillon⁴, Doug Wiczorek¹, Céline Ménager², Eric B. Vincent¹ and Trista Schagat¹

¹Promega Corporation, 2800 Woods Hollow, Madison WI, USA 53711, ²Promega Europe Training and Applications Laboratory (PETAL), 24 Chemin des Verrières, Charbonnières-les-Bains, France 69260, ³International Plant Analysis and Diagnostics srl., Loc. Cascina Codazza, Via Einstein, Italy 26900, ⁴Qualiplante, Cap Alpha, Avenue de l'Europe, Clapiers, France 34830.



1. Introduction

The diverse range of plant species and tissue types found in nature present a unique set of obstacles for nucleic acid isolation from botanical samples. In addition, the carry-over of substances present in certain plants can interfere with quantitation and downstream amplifications. These difficulties highlight the need for robust, reliable chemistries for molecular biology studies with plants. We present several solutions to purify and amplify nucleic acid from a variety of plant types and tissues with a focus on agriculturally relevant applications.



2. Methods: A Complete Nucleic Acid Toolbox for Plant Samples

Flexible, effective workflow options for nucleic acid purification, quantitation, and amplification from botanical samples. Tested for a variety of plant types and tissues.

Homogenize

Purify

- ReliaPrep™ MiniPreps
- Maxwell® RSC
- High throughput options

Quantify

Fluorescence-based quantification of extracted RNA with Quantus™/GloMax® instruments

Analyze

- qPCR
- RNA sequencing
- Gel electrophoresis

Find Your Plant Type using the Applications Selector Tool

For protocols and example data for a number of diverse plant types visit the applications selector at Promega.com.

Quantify:

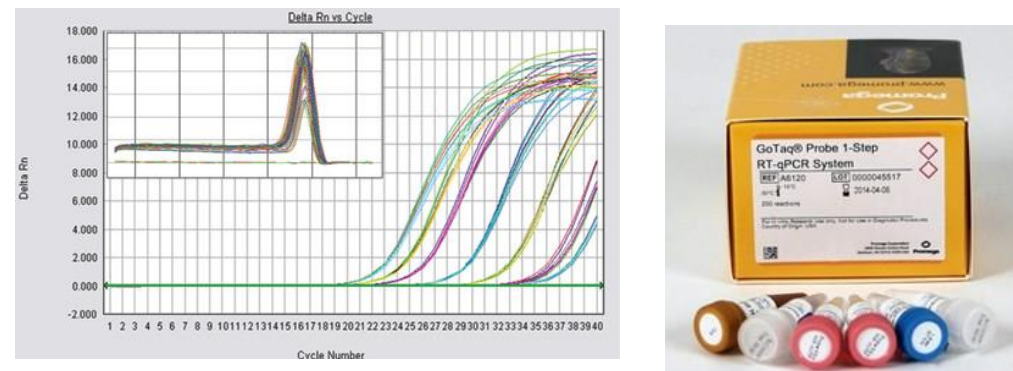
Quantifluor® System

- Dye Based Quantitation**
- High sensitivity
 - Accurate
 - dsDNA, ssDNA, or RNA

Amplify:

GoTaq® Systems

- Assay flexibility:**
- qPCR or RT-qPCR
 - 1-Step or 2-Step RT
 - Probe or dye
 - Fast or standard cycling

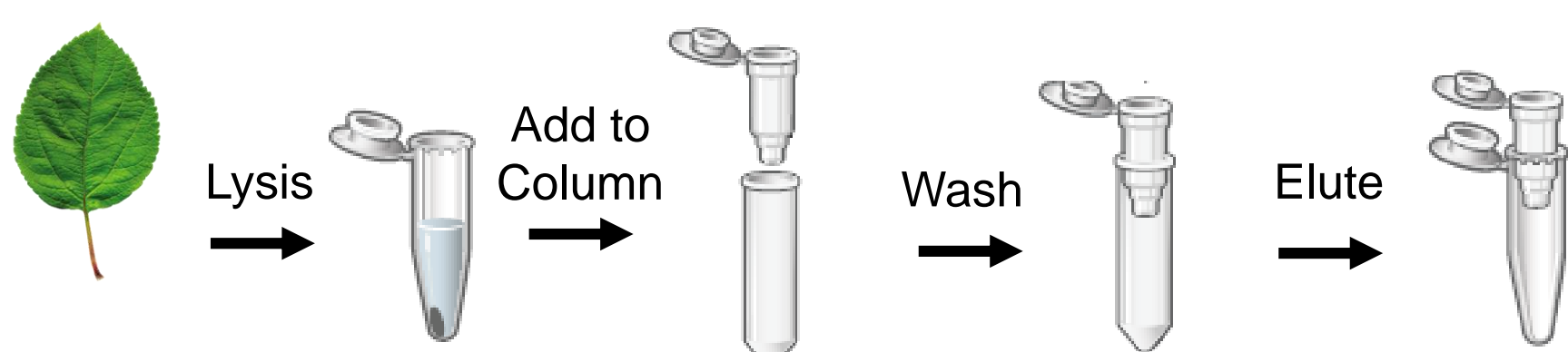


3. A Manual Nucleic Acid Isolation From Three Species of Plant Leaves

ReliaPrep™ Manual Nucleic Acid Purification Systems for Plant Samples

RNA: ReliaPrep™ RNA Tissue Miniprep System
DNA: ReliaPrep™ gDNA Tissue Miniprep System

Method: A Simple Spin-Column Based Workflow

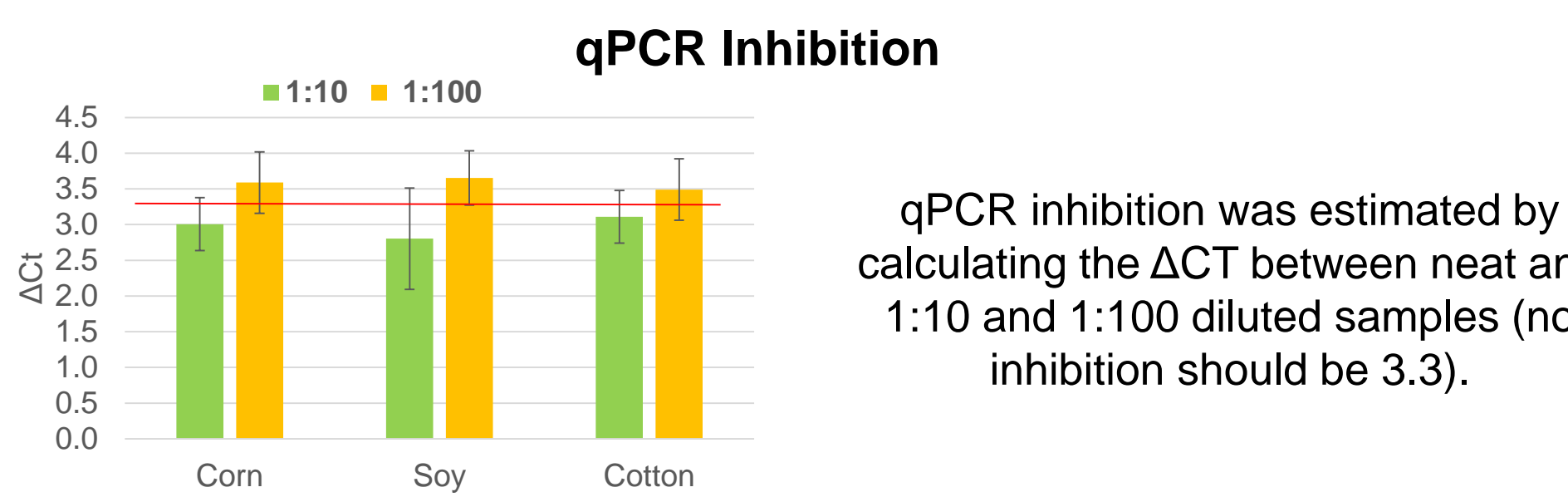
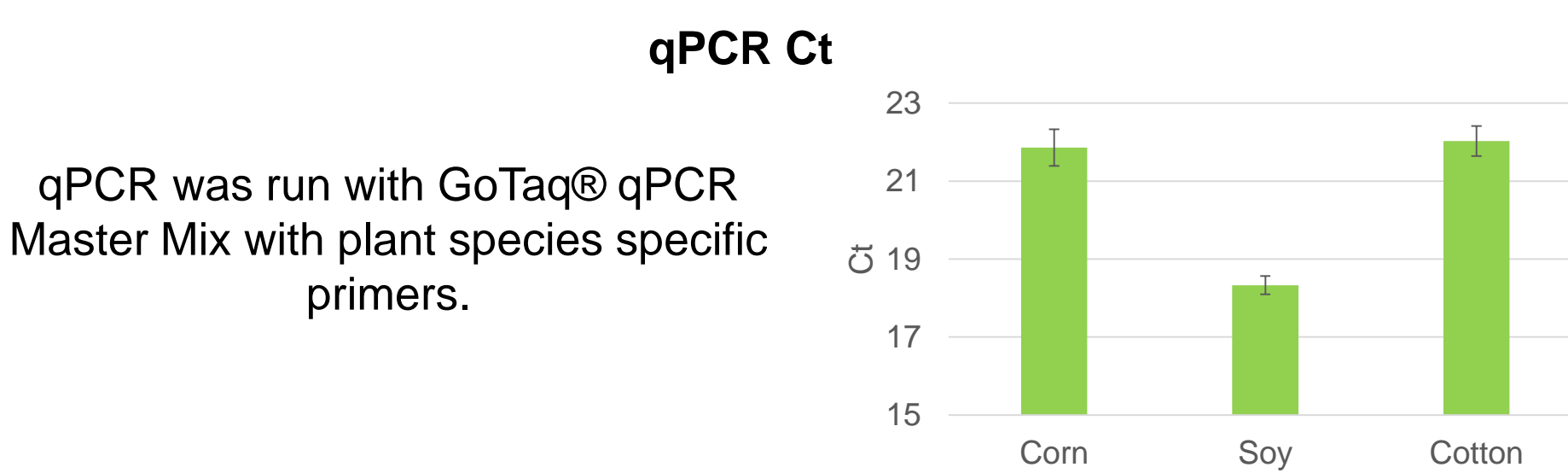
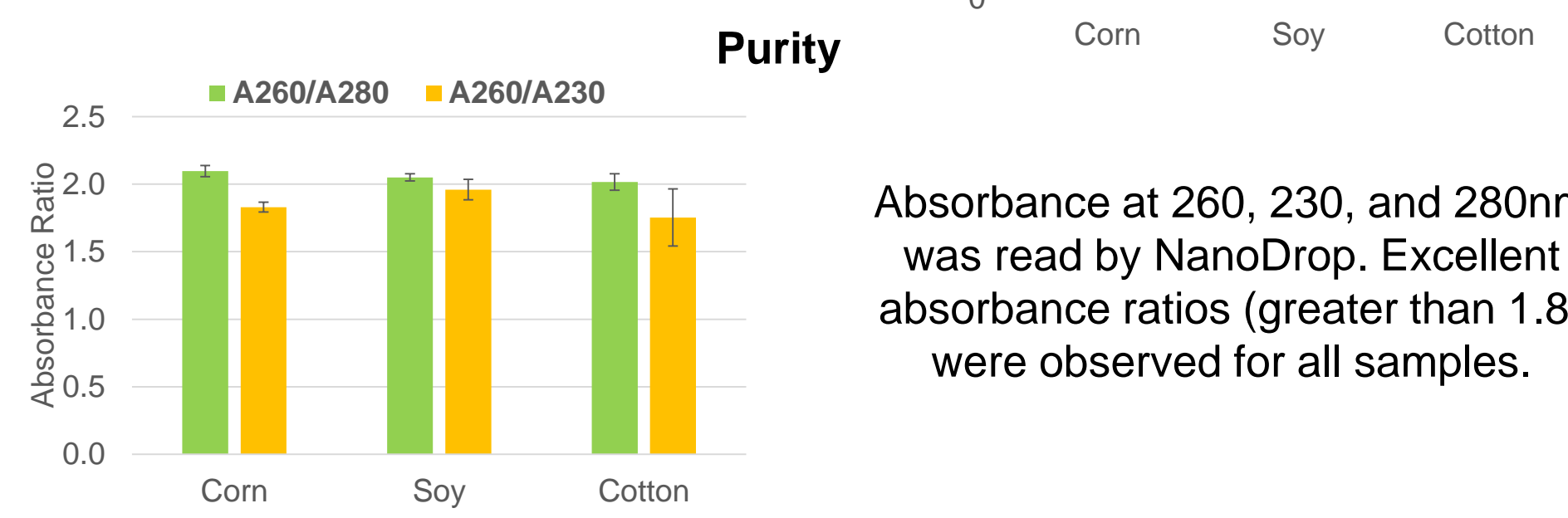
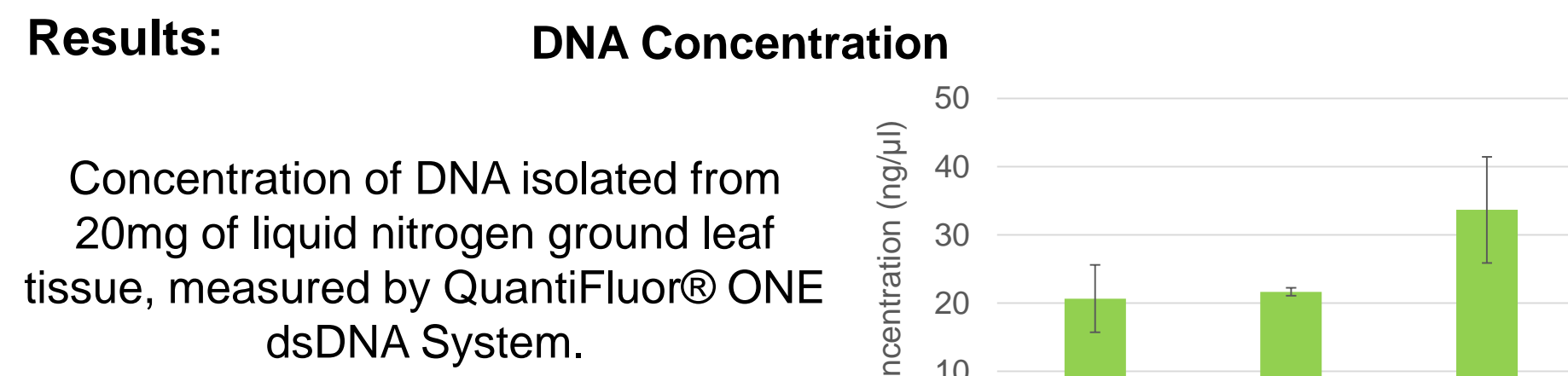


Sample: Three species of plant leaves



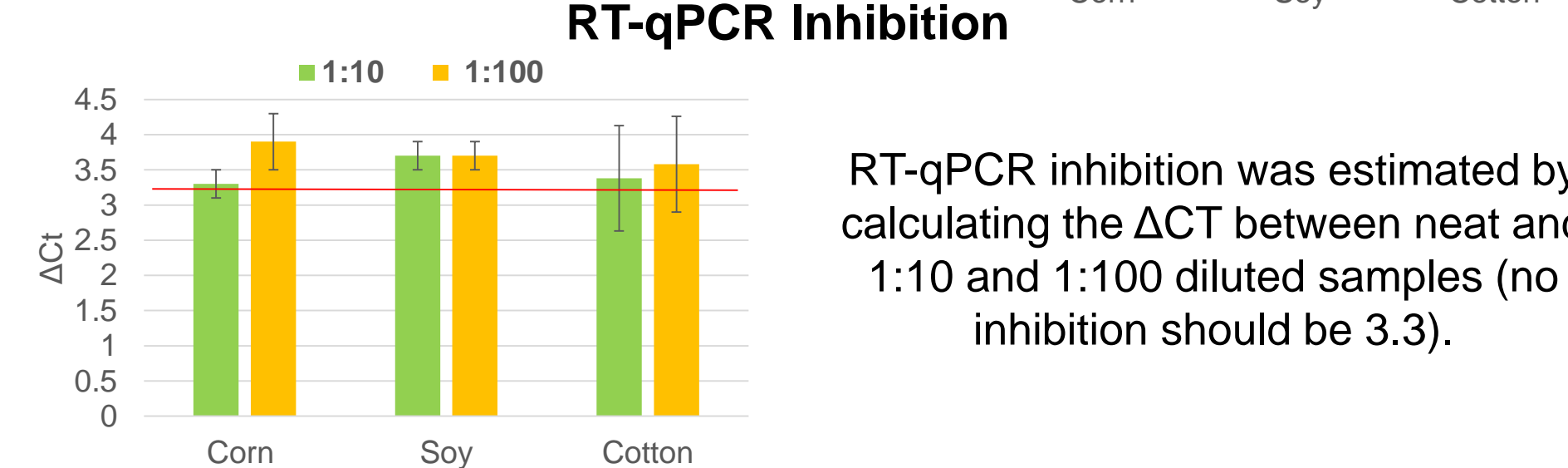
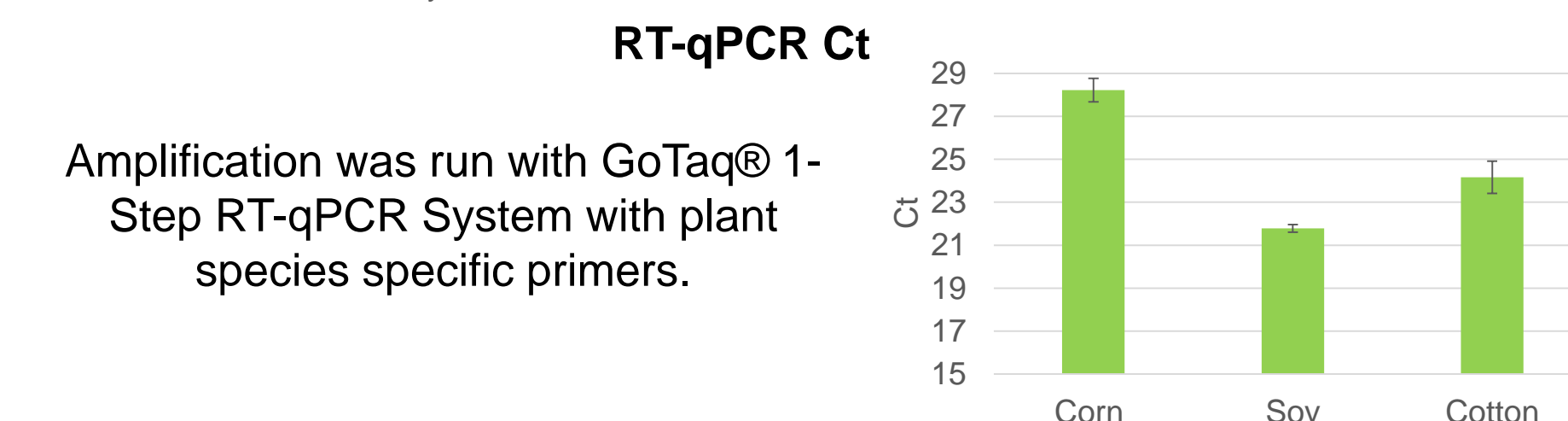
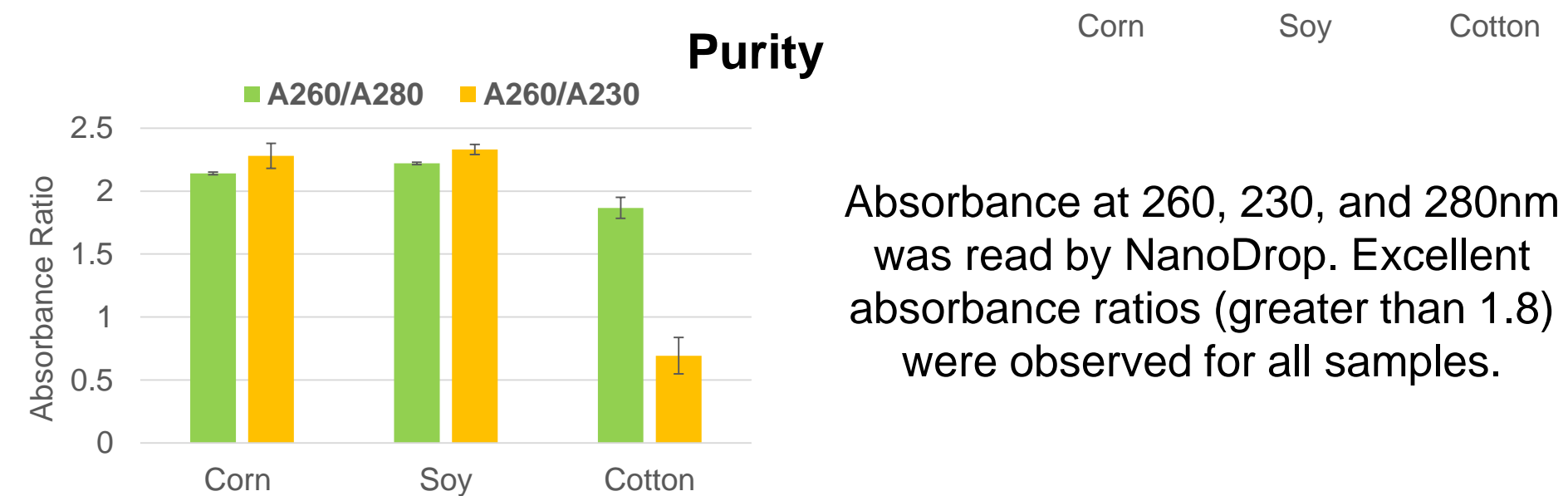
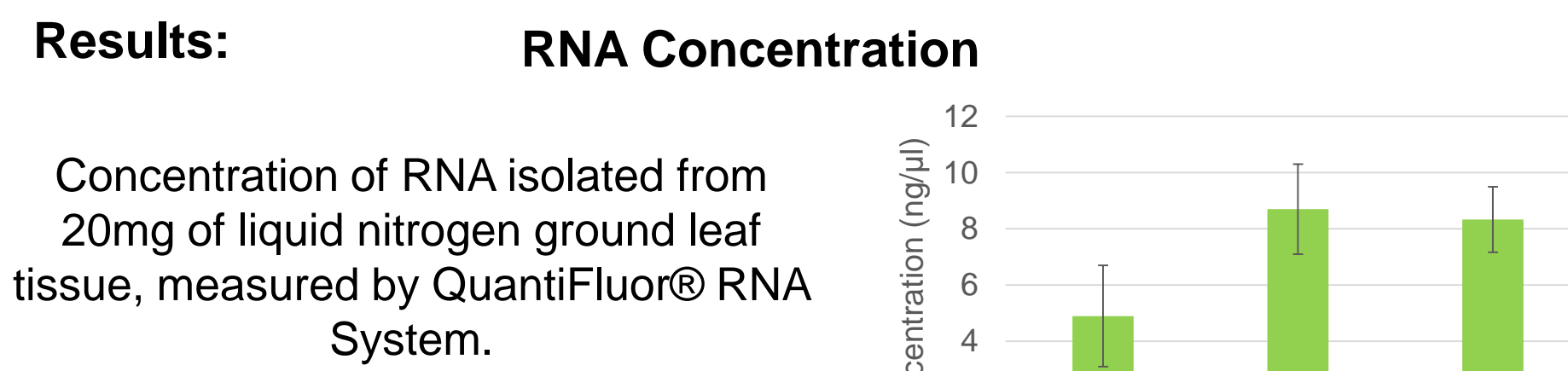
4. Manual DNA Isolation From Three Species of Plant Leaves

Results:



5. Manual RNA Isolation From Three Species of Plant Leaves

Results:

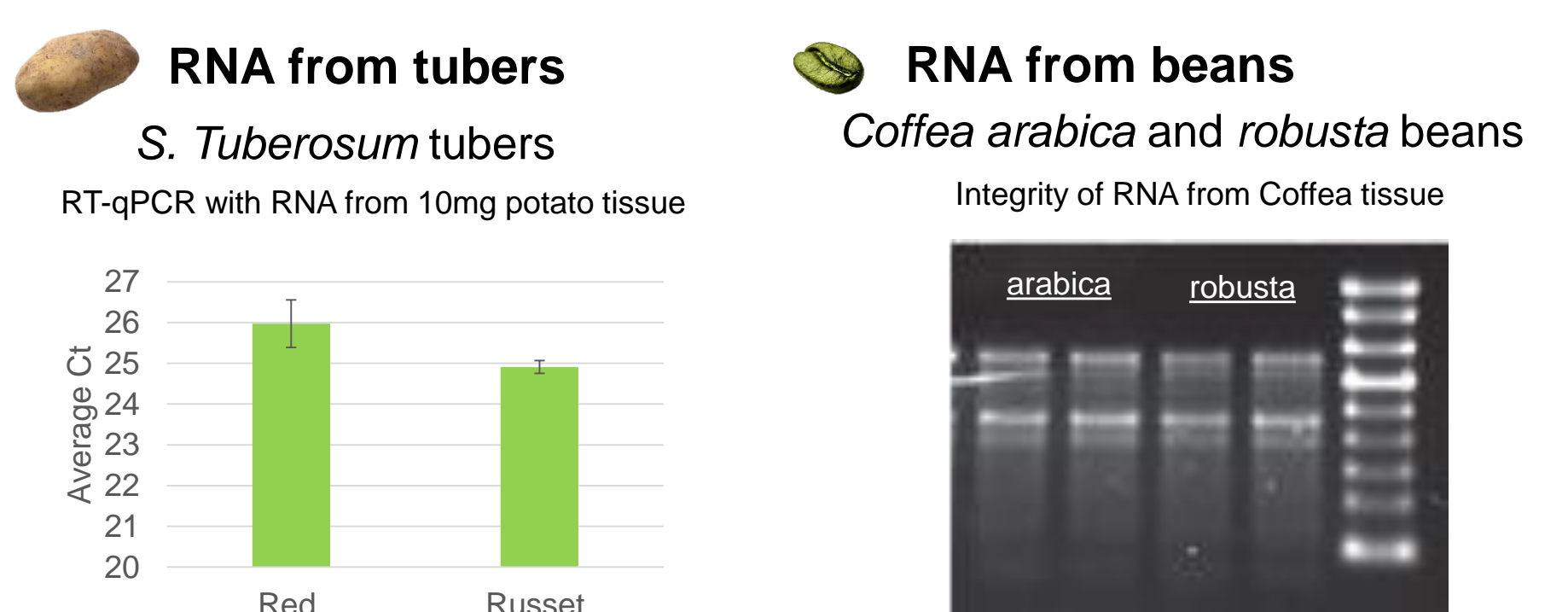
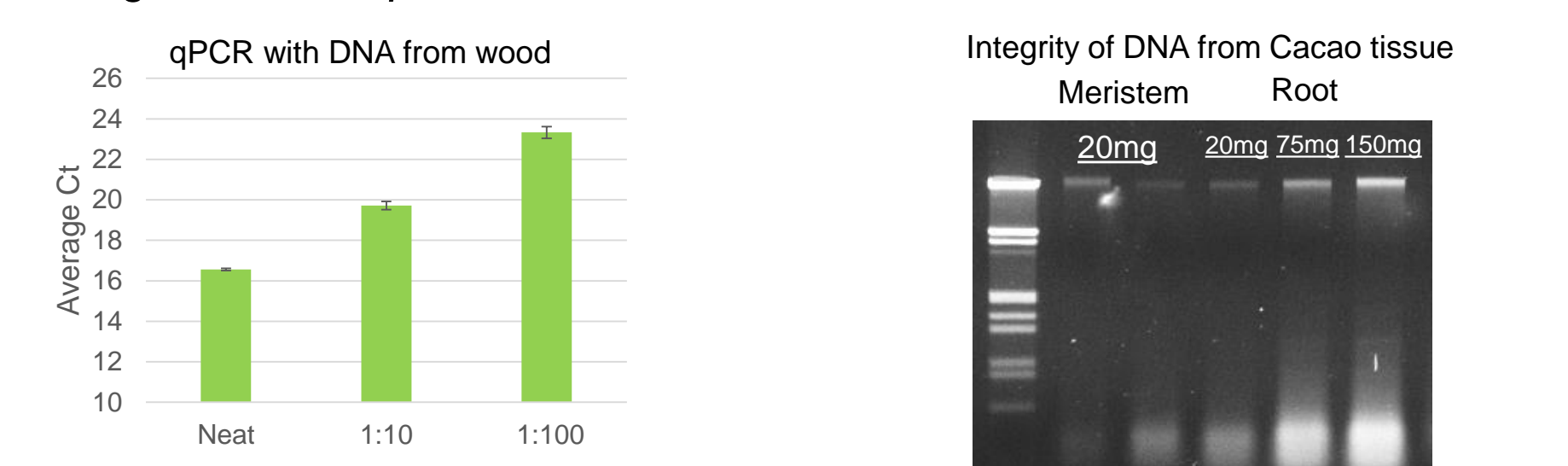


6. Nucleic Acid Extraction from Diverse Botanical Tissue Types

We have optimized a number of protocols to obtain amplifiable nucleic acid from difficult and diverse plant tissues.

DNA from branch tissue
100mg of *Prunus sp.* branch tissue

DNA from meristem and root
Theobroma cacao meristem and root



7. RNA Isolation from Virus in Plant Tissues

Viral RNA Isolation From Seeds

Methods: Spike Tobacco Mosaic Virus into seed lysates and purify RNA using the Maxwell® RSC Plant RNA Kit for viral detection with RT-qPCR.

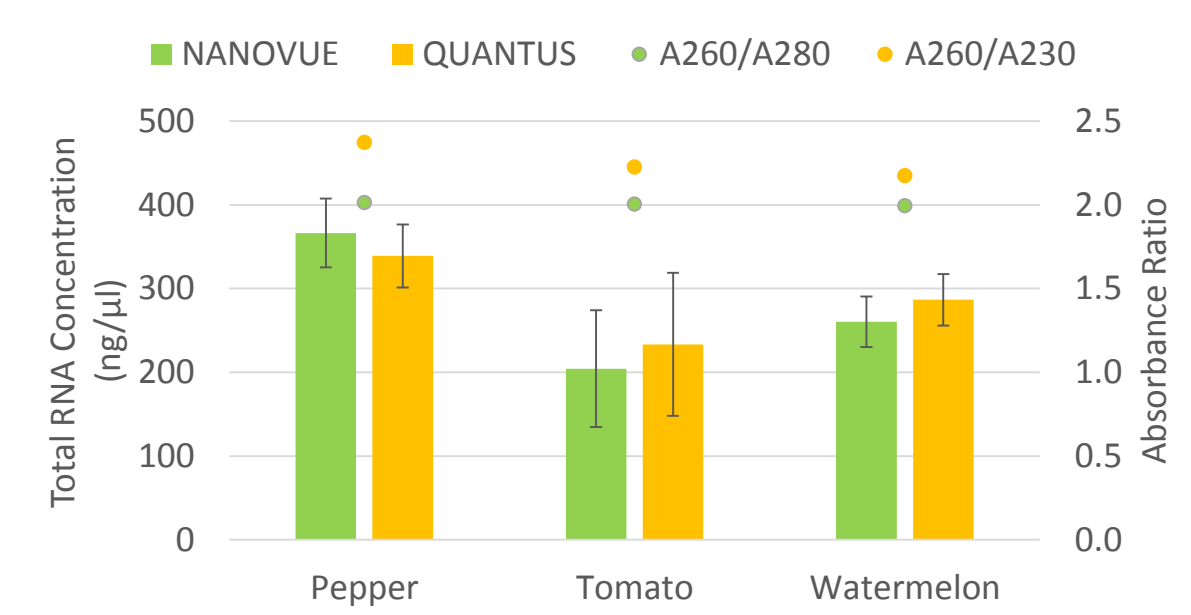
Sample: Three species of plant seeds



Results:

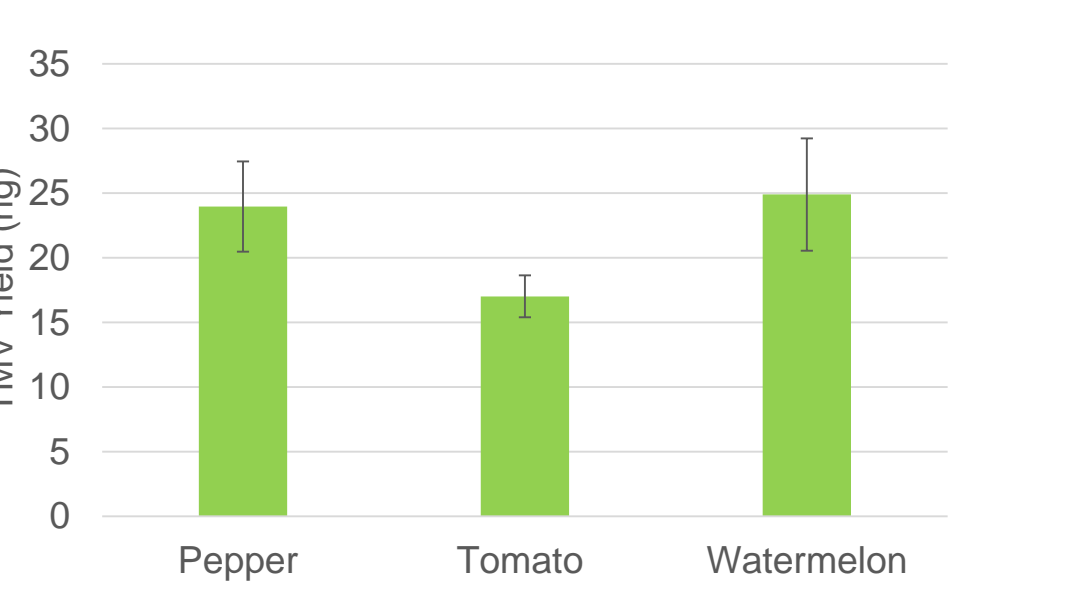
- Concentrated, pure RNA isolation from plant seeds
- Excellent >80% recovery of spiked Tobacco Mosaic Virus RNA

Total RNA Concentration and Purity



Sample Type	% Viral Recovery
Pepper	119.8
Tomato	85.0
Watermelon	124.5

Viral RNA quantification by RT-qPCR



8. Viroid RNA Extraction From Citrus Leaves

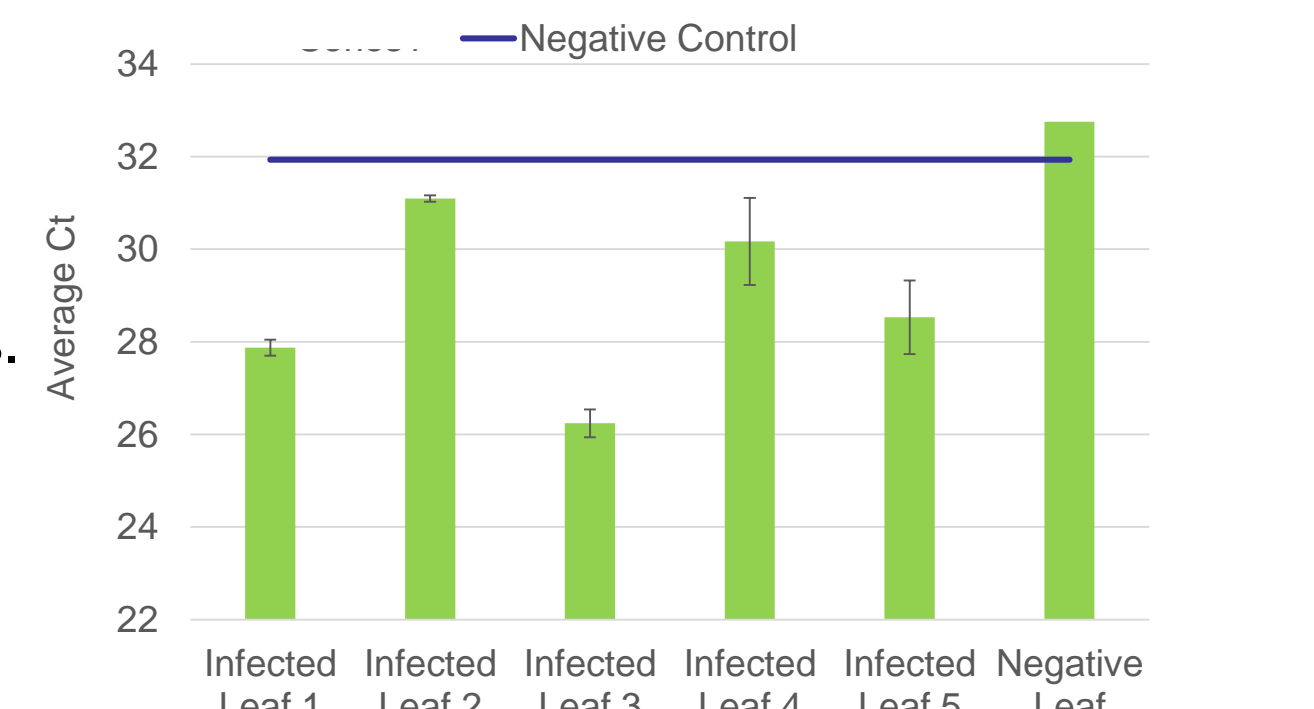
Methods: Extraction of Citrus exocortis viroid (CEVd) RNA from infected lemon leaves for viral detection with Qualiplante CEVd One-Step Real-Time RT-PCR Assay. The small size of this viroid RNA requires use of the Maxwell® RSC miRNA Kit for best viral recovery.

Sample: Leaves from infected and healthy lemon trees, kindly provided by International Plant Analysis and Diagnostics Srl.

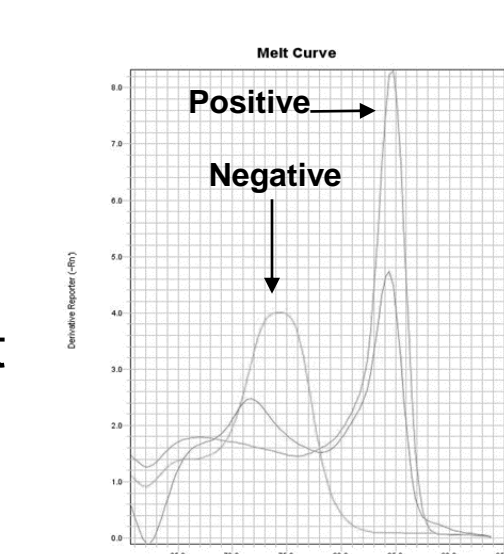


Results: Viroid RNA amplification by CEVd specific RT-qPCR

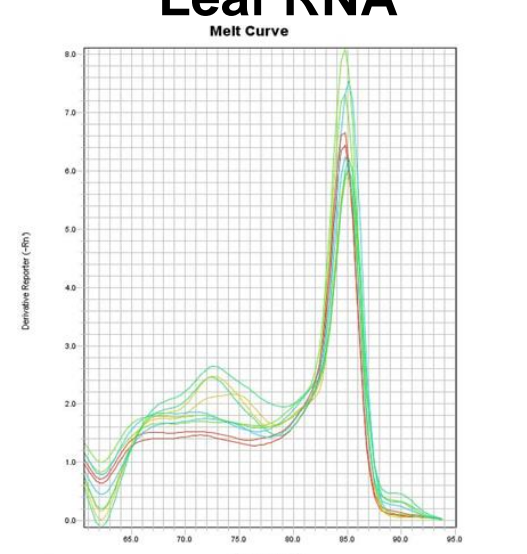
- Amplifiable CEVd Viroid RNA was isolated from all infected leaf samples.
- RNA isolated from a CEVd negative leaf did not amplify below negative control Ct values.



Melt Curve of Controls



Melt Curve of Infected Leaf RNA



- RNA isolated from infected leaf samples produced specific melt curves with the CEVd assay.

9. Summary

Conclusions:

- Nucleic acid can be isolated from plants in three flexible formats:
 - Manual/low throughput: ReliaPrep™ Miniprep Systems
 - Low/medium throughput: Maxwell® RSC Instrument and Kits
 - High throughput: Maxwell® HT
- Downstream quantitation and amplification products compliment this plant based workflow:
 - Quantifluor® fluorescent dye-based nucleic acid quantification
 - GoTaq® amplification reagents
- Nucleic acid isolation from a number of plant leaves, branches, meristems, roots, beans, and seeds have been tested with Maxwell® and ReliaPrep™ Systems
- Viral RNA from infected leaves and seeds can also be isolated

Resources: Purification Solutions for Common Plant Tissue Types

Sample	Nucleic Acid	ReliaPrep™ System	Maxwell® RSC	Maxwell® HT
Leaf	DNA	ReliaPrep™ gDNA Tissue Miniprep System	Maxwell® RSC Plant DNA Kit	Custom Options Available
	RNA	ReliaPrep™ RNA Tissue Miniprep System	Maxwell® RSC Plant RNA Kit	
	miRNA	ReliaPrep™ miRNA Miniprep System	Maxwell® RSC miRNA Kit	
Seed	DNA	ReliaPrep™ gDNA Tissue Miniprep System	Maxwell® RSC Plant DNA Kit	Custom Options Available
	miRNA	ReliaPrep™ miRNA Miniprep System	Maxwell® RSC miRNA Kit	

* Due to the diversity present in plants these are suggestions, and results may vary. Contact Promega Technical Services with any questions

For additional Maxwell® or ReliaPrep™ information, visit:

- Maxwell® : <http://www.promega.com/products/instruments/maxwell-systems/>
- ReliaPrep™ : <http://www.promega.com/products/pm/rna-purification-from-cells-and-tissues/>