Development of Automated DNA and RNA Plant Extraction Kits using the new Maxwell® RSC Platform

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1. Introduction

The wide diversity of plants makes it difficult to find universal and robust solutions for extracting DNA and RNA. Poor and inconsistent extraction methods can carry over secondary compounds such as phenols and carbohydrates resulting in inaccurate quantification and inhibition of downstream amplification analyses. With this challenge in mind, Promega has developed an automated system that provides consistent DNA and RNA extraction from a variety of plant tissue samples.

The DNA extraction protocol was specifically optimized for purifying high molecular weight DNA, balancing the need for maximizing DNA yield per sample while minimizing multiple types of qPCR and next-generation sequencing inhibitors. Likewise, the RNA extraction protocol was optimized to prevent degradation of RNA, improve purity, minimize RT-qPCR inhibitors, and maximize yield.

Both the RNA and DNA Plant kits require a minimal amount of sample input for the purification of high quality nucleic acids and are compatible with common downstream amplification-based assays.

While optimizing these protocols, we also discovered a pitfall in using spectrophotometers for the quantification of nucleic acids from particular plant species.

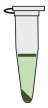
2. Maxwell® RSC Instrument

- Purifies 1 to 16 samples of DNA in less than 40 minutes.
- Purifies 1 to 16 samples of RNA in less than 50 minutes.
- Controlled by software user interface on a Windows 7 touchscreen tablet
- Integrated quantitation with Quantus™ Fluorometer

3. Plant Kit Workflow

DNA

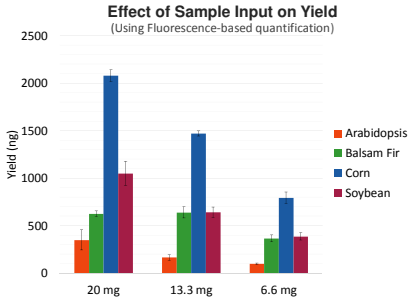
1. Grind sample under liquid nitrogen or bead beater
2. Add buffer. Vortex
3. Centrifuge max speed
4. Add clear lysate to the prepared cartridge
5. Load cartridges into the RSC and hit 'Run'.

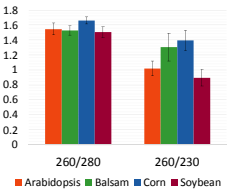


RNA

1. Grind sample under liquid nitrogen or bead beater
2. Add buffer. Vortex
3. Briefly homogenize tissue
4. Add lysis buffer
5. Let sit at RT for 10 minutes
6. Centrifuge at max speed
7. Add clear lysate to the prepared cartridge.
8. Load cartridges into the RSC and hit 'Run'.

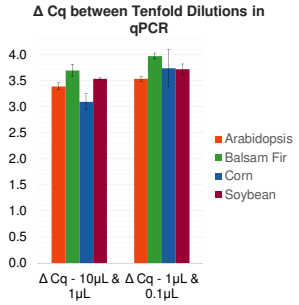
4. DNA Yield and Purity



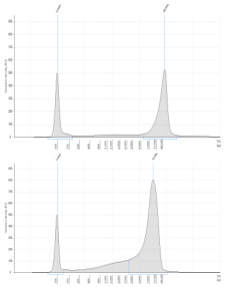


The DNA Plant Kit has been successful at isolating DNA from the leaves of:
Amelanchier, apple, apricot, cacao, cherry, coffee, date palm, eggplant, fig, goji, gooseberry, grape, kiwi, lemon, mandevilla, medlar (Holland and Spanish), milkweed, mulberry, mustard greens, oil palm, peach, pear, peony, persimmon, plum, potato, privet, quince, raspberry, red Russian kale, rice, rose, rosemary, sage, *stapelia*, strawberry, thyme, tomato, zucchini

5. DNA Size & Amplification



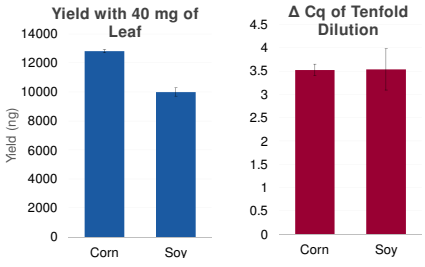
Since 10X dilutions should amplify ~3.3 cycles apart in a perfectly efficient qPCR assay, the ΔCq generated by a dilution series can be used to detect inhibitory contaminants in the eluates.



RSC Plant DNA method produces high molecular weight gDNA.

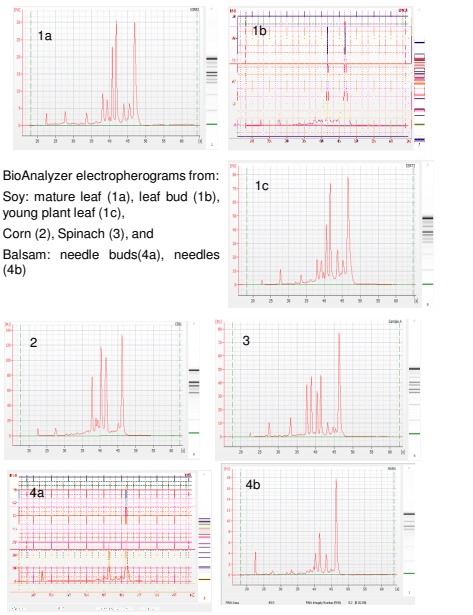
Agilent TapeStation electropherograms show fragment lengths peaking at (a) 10,940 bp for corn, (b) 56,845 bp for soy, and 30,122 bp for arabidopsis (not shown).

6. RNA Yield and Amplification



The RNA Plant Kit has been successful at isolating RNA from the leaves of:
Amelanchier, apple, apricot, cherry, coffee, eggplant, fig, goji, gooseberry, grape, kiwi, lemon, mandevilla, medlar (Holland and Spanish), milkweed, mulberry, oil palm, peach, pear, peony, persimmon, plum, potato, privet, quince, raspberry, rice, rose, *stapelia*, strawberry, tomato, zucchini

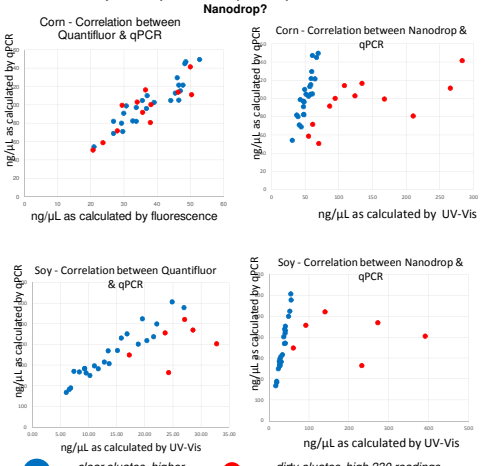
7. RNA Quality



BioAnalyzer electropherograms from:
Soy: mature leaf (1a), leaf bud (1b), young plant leaf (1c),
Corn (2), Spinach (3), and
Balsam: needle buds(4a), needles (4b)

8. Spectrophotometry vs. Fluorescence

Which assay is more predictive of qPCR amplification—Quantifluor or Nanodrop?



= *clear eluates, higher 260/230*

= *dirty eluates, high 230 readings. Caused by transferring part of the pellet into the cartridge.*

Both the fluorescence and UV-absorbance based quantification of the sample correlate well with qPCR amplification in clean eluates. However, fluorescence better estimates concentration in samples with high 230 nm readings.

9. Conclusion

Advantages of the RSC Instrument:

- Quickly and simultaneously purify up to 16 plant tissue samples without organic solvents
- Automated extraction of gDNA or RNA from plants frees up researchers' time
- Low %CV between replicates and run-to-run consistency.

Advantages of the Plant RNA and Plant DNA kits:

- Isolation method is compatible with a broad panel of species
- Efficient nucleic acid capture (into the microgram range) minimizes the need for re-isolation or pooling of samples
- Isolated DNA is high genomic weight
- Isolated RNA is high integrity
- Purifies amplifiable DNA and RNA with minimal co-purification of inhibitors.

Product Information:

- Instrument used is the Maxwell® RSC instrument (Promega Catalog #AS4500)
- For further technical information about these chemistries, contact Promega Technical Services at 1-800-356-9526, or at techserv@promega.com.