A Comparison of Methods for DNA and RNA Purification from Plant

A Maxwell® RSC Instrument Application Note

Materials Required:

- Maxwell® RSC Plant DNA Kit (Cat.# AS1490)
- Maxwell® RSC Plant RNA Kit (Cat.# AS1500)
- QuantiFluor® dsDNA System (Cat.# E2670)
- QuantiFluor® RNA System (Cat.# E3310)
- GoTaq® qPCR Master Mix (Cat.# A6001)
- GoTaq® 1-Step RT-qPCR System (Cat.# A6020)

Instrument Requirements:

- Maxwell® RSC (Cat.# AS4500)
- NanoDrop® 1000 (Agilent)

Performance Comparison:

- DNeasy® Plant Mini Kit (Qiagen, Cat.# 69104)
- RNeasy® Plant Mini Kit (Qiagen, Cat.# 74903)

Automated purification of DNA or RNA from plant leaf tissue using the Maxwell® RSC Instrument.

Introduction

The Maxwell® Rapid Sample Concentrator (RSC) Instrument is an automatic nucleic acid purification system that will process up to 16 samples in a single run. Used in combination with prefilled reagent cartridges, the Maxwell® RSC can purify DNA or RNA from a wide range of sample types. The intuitive graphical user interface makes the instrument easy to use, and the integrated Quantus™ Fluorometer lets you collect purification and quantification data in one report.

The Maxwell® RSC Plant DNA and RNA Kits provide a simple method for purifying nucleic acid from plant leaf tissue samples on the Maxwell® RSC Instrument. Here, we compare the performance of the Promega Maxwell® RSC Plant Kits with the Qiagen DNeasy® and RNeasy® Plant Mini Kits.

Methods

DNA purification was performed using 20mg of corn, soy or Arabidopsis leaf tissue with either the Maxwell® RSC Plant DNA Kit or Qiagen DNeasy® Plant Mini Kit. Samples were homogenized by bead-beating as recommended in the technical manuals. Equivalent elution volumes (50µl) were used for both kits. All conditions were performed in triplicate. DNA concentration and yield were measured by dye-based quantitation using the QuantiFluor® dsDNA System (Cat.# E2670) with the integrated Quantus™ Fluorometer (Cat.# E6150). Purity was measured by absorbance on a NanoDrop® 1000 Instrument. Real-time amplification was performed with plant DNA species-specific primers using GoTaq® qPCR Master Mix (Cat.# A6001).

RNA was purified from 50mg of liquid-nitrogen-ground corn, soy, or Arabidopsis leaf tissue with either the Maxwell® RSC Plant RNA Kit or Qiagen RNeasy® Plant Mini Kit. Equivalent elution volumes (50µl) were used for both kits. All conditions were performed in triplicate. RNA concentration and yield were measured by dye-based quantitation with the QuantiFluor® RNA System (Cat.# E3310) on the integrated Quantus™ Fluorometer. Purity was measured by absorbance on a NanoDrop 1000 Instrument. RT-qPCR was performed with plant species-specific primers using GoTaq® 1-Step RT-qPCR System (Cat.# A6020).

Results

DNA Yield and Purity

DNA yield from each plant leaf tissue is shown in Figure 1, Panel A for both purification methods. Equivalent or greater DNA yields were obtained using the Maxwell® RSC Plant DNA Kit when compared to DNeasy® for all three plant species. A_{260}/A_{280} and A_{260}/A_{230} ratios are shown in Figure 1, Panel B for both purification methods. The two kits had similar A_{260}/A_{280} ratios, however the DNeasy kit exhibited a higher A_{260}/A_{230} ratio for corn and soy samples along with greater variability.

DNA qPCR Performance

Cq values were obtained from qPCR with plant leaf tissue DNA using GoTaq® qPCR Master Mix (Figure 1, Panel C). Samples isolated with the Maxwell® RSC were amplified with lower or equivalent Cq values when compared to DNeasy® purified samples. No significant PCR inhibition was observed with any of the plant DNA samples tested (data not shown). Plant DNA purified with the Maxwell® RSC Plant DNA Kit performed well in amplification-based applications.

RNA Yield and Purity

RNA yield was obtained from each plant leaf tissue using the two methods is shown in Figure 2, Panel A. The Maxwell® RSC Plant RNA Kit and the RNeasy® Kit recovered equivalent RNA yields for Arabidopsis and soy. A_{260}/A_{280} and A_{260}/A_{230} ratios are shown in Figure 2, Panel B. All ratios were above 1.7 for Maxwell® RSC Plant RNA Kit samples, indicating excellent purity.

RNA RT-qPCR Performance

Figure 2, Panel C shows the results of RT-qPCR analysis using RNA purified with each method. Samples isolated with the Maxwell® RSC System had lower or equivalent Cq values compared to RNeasy® purified samples. No PCR inhibition

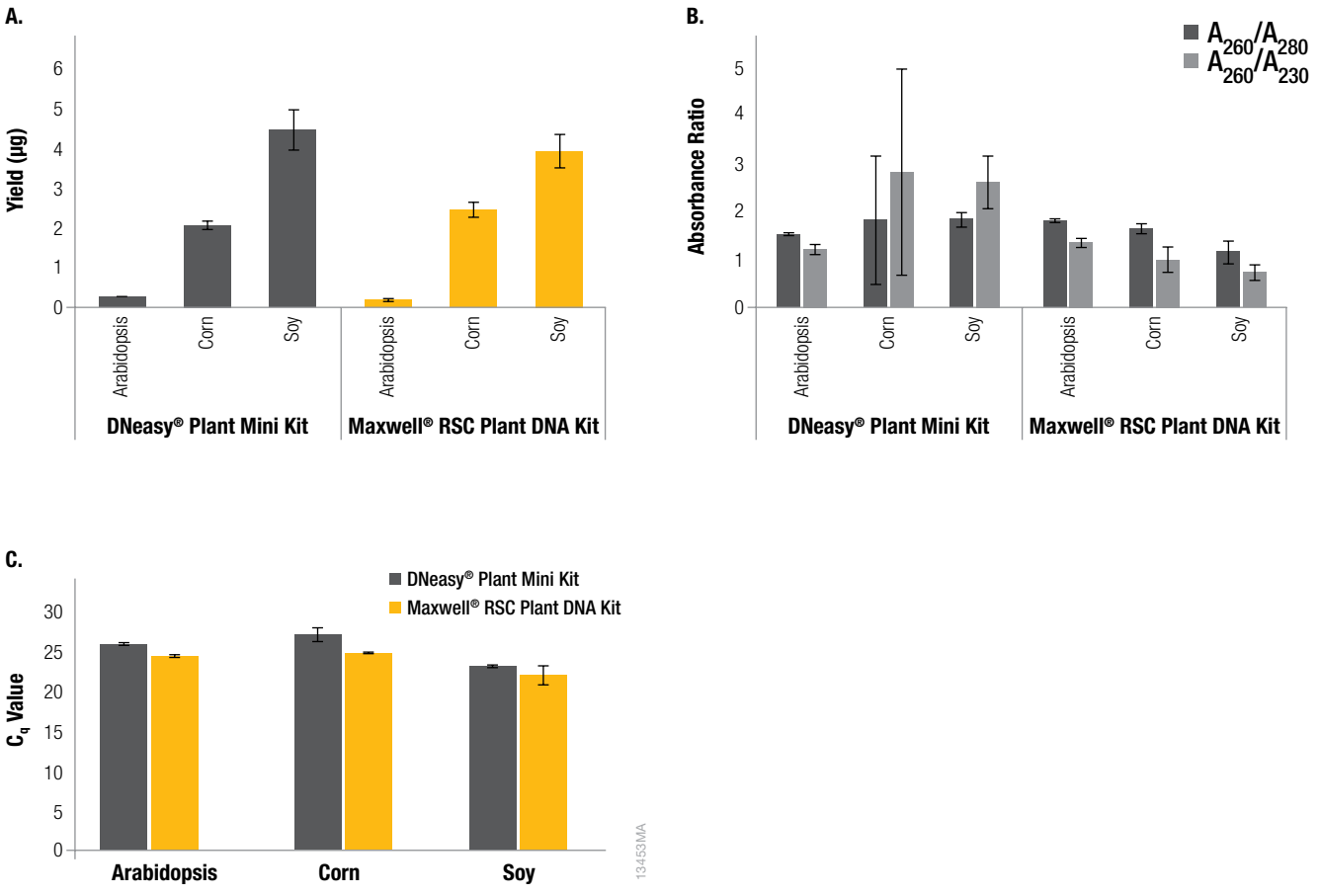

Figure 1. Analyses of DNA eluates isolated from plant leaves using two nucleic acid chemistries. Panel A. DNA yield from plant leaf tissues. DNA quantitation was performed using 1µl of purified plant DNA with the QuantiFluor® dsDNA System on the Quantus™ Fluorometer. **Panel B.** Absorbance ratios of DNA from plant leaf tissue. Absorbance ratios was measured on a NanoDrop® 1000 Instrument. **Panel C.** Cq values from qPCR with DNA from plant leaf tissue. qPCR was performed using 1µl of purified plant DNA with the GoTaq® qPCR Master Mix in a 50µl reaction. Data represent the mean ± standard deviation for n=3 replicate samples from each plant tissue and condition.

was seen with samples purified with either method (data not shown). These results show that plant RNA purified with the Maxwell® RSC Plant RNA Kit is suitable for amplification-based applications.

Conclusions

The Maxwell® RSC results matched or outperformed Qiagen DNeasy® and RNeasy® purified samples in every parameter tested except for RNA yield from corn (Figure 2. Panel A). The two kits had similar A_{260}/A_{280} ratios, indicating minimal contamination from plant phenolic compounds. The DNeasy® and RNeasy® kits exhibited higher variability in A_{260}/A_{230} ratios; co-purified carbohydrates are a common reason for this ratio to be lower in plants. The Maxwell® RSC Plant Kits amplified nucleic acids with equivalent or lower Cq values than both Qiagen kits.

In addition to the excellent performance, the Maxwell® RSC Plant DNA and RNA Kits provide workflow benefits as well. The Maxwell® RSC Plant DNA and RNA Kits have a short 5-15 minute preprocessing step followed by automated purification. This semi-automated format saves hands-on time and reduces potential user error. The Maxwell® RSC Plant DNA and Maxwell® RSC Plant RNA Kits are an efficient, low to medium through-put, alternative to manual spin-based procedures for isolating pure, high quality nucleic acid from plant leaf tissue samples.

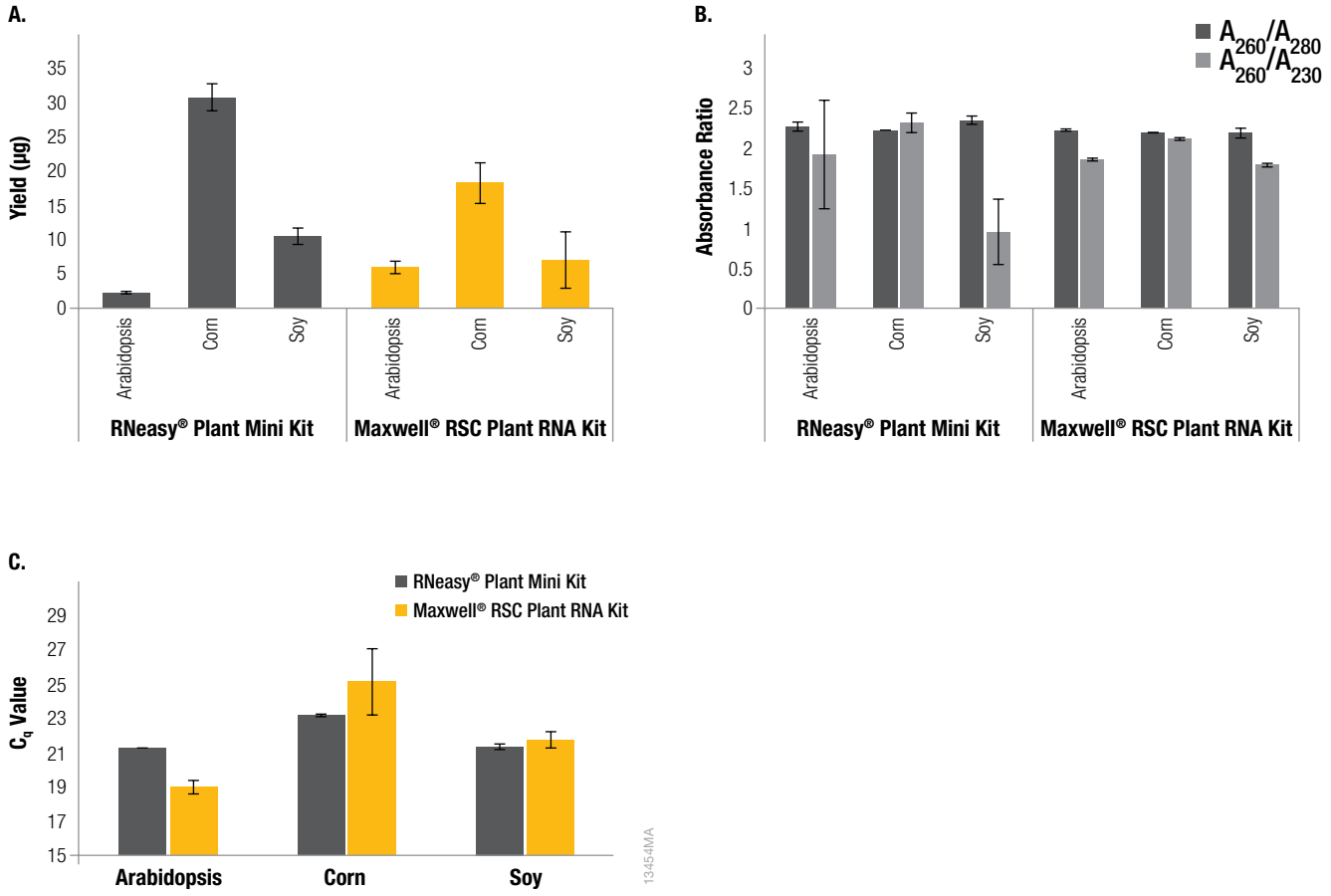

Figure 2. Analysis of RNA from plant leaves using two nucleic acid chemistries. Panel A. RNA yield from plant leaf tissues. RNA quantitation was performed using 1µl of purified plant RNA with the QuantiFluor® RNA System on the integrated Quantus™ Fluorometer. **Panel B.** Purity of RNA from plant leaf tissue. Absorbance was measured on a NanoDrop® 1000. **Panel C.** Cq values from RT-qPCR with RNA from plant leaf tissue. 1µl of purified plant RNA was used in a 50µl RT-qPCR reaction with the GoTaq® 1-Step RT-qPCR System. Data shown is the mean ± standard deviation for n=3 replicate samples from each plant leaf tissue and condition.

Ordering Information

Product	Cat.#
Maxwell® RSC Instrument	AS4500
Maxwell® RSC Plant DNA Kit	AS1490
Maxwell® RSC Plant RNA Kit	AS1500
QuantiFluor® dsDNA System	E2670
QuantiFluor® RNA System	E3310
GoTaq® qPCR Master Mix	A6001
GoTaq® 1-Step RT-qPCR System	A6020

PROMEGA CORPORATION • 2800 WOODS HOLLOW ROAD • MADISON, WI 53711-5399 USA • TELEPHONE 608-274-4330
www.promega.com • ©2015 PROMEGA CORPORATION • ALL RIGHTS RESERVED • PRICES AND SPECIFICATIONS SUBJECT TO CHANGE WITHOUT PRIOR NOTICE • PRINTED IN USA 12/15 • 22551298 • PART #AN291