Promega

Product Application

DNA Extraction from Rice Leaves using the Maxwell® RSC System

Isolate high-quality, amplifiable DNA from rice leaves using the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Plant DNA Kit. (Cat.# AS1490)

Analyses: GoTaq® qPCR, QuantiFluor®ONE dsDNA System and NanoDrop® One quantitation

Sample Type(s): Rice leaves

Input: 20mg

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.

For further information, see Technical Manual TM458, available at: www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® RSC Plant DNA Kit. (Cat.# AS1490)
- Maxwell® RSC Instrument (Cat.# AS4500)
- mortar and pestle (or bead-beating device)
- microcentrifuge
- 1.5ml tubes

Protocol:

1. Cut the leaf tissue into small pieces.
2. Transfer to a mortar and grind the leaf.
   Note: Sample homogenization can also be performed using a bead-beating device following manufacturer recommendations.
3. Weigh 20mg of homogenized leaf tissue and transfer into a 1.5ml tube for each sample.
4. Add 300µl of Tail Lysis Buffer (TLA) to each sample. Vortex for 15 seconds.
5. Add 10µl of RNase A to each sample. Vortex for 10 seconds.
6. Centrifuge tubes for 2 minutes at 12,000 × *g*.
7. Place the necessary number of Maxwell® RSC Plant DNA cartridges into the deck tray(s).
8. Add 300µl of Nuclease Free Water to well #1 of each cartridge.
9. Transfer 200µl of each plant lysate sample into well #1 of the cartridges. Take care not to transfer any solid particles.
10. Place the supplied elution tubes into the sample rack, and add 50µl of the supplied elution buffer.
11. Place the plungers into well #8 of the cartridges.
12. Select the Plant DNA method on the Maxwell® RSC Instrument and start run.

Results:

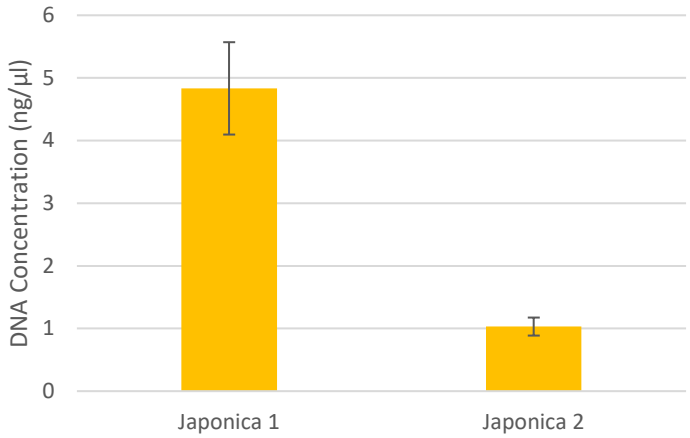

Figure 1. Concentrations of DNA purified from 20mg of homogenized rice leaves using the Maxwell® RSC Plant DNA Kit. Samples were eluted in 50µl. QuantiFluor® ONE dsDNA System (Cat.# E4870) was used for quantitation. Leaves were from 2 different Japonica rice plants. Mean DNA concentration ± standard deviation of N=3 is shown.

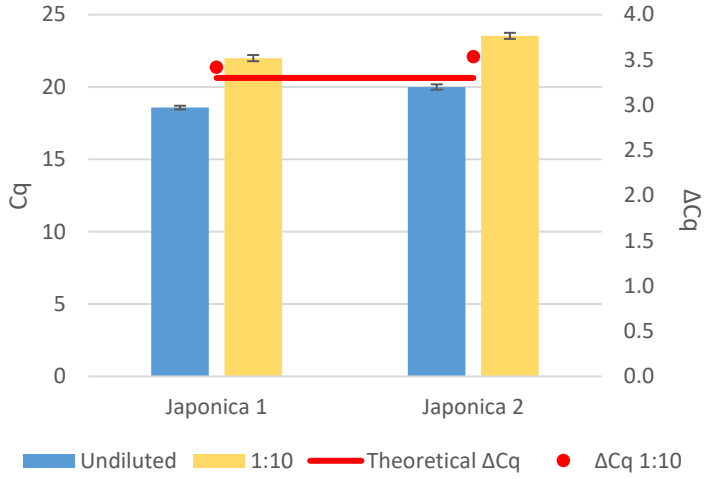

Figure 2. qPCR analysis of DNA purified from 20mg of homogenized rice leaves using the Maxwell® RSC Plant DNA Kit. Mean Cq and ΔCq values ± standard deviation for 2µl of undiluted and 1:10 diluted rice leaf DNA amplified using GoTaq® qPCR Master Mix (Cat.# A6002) and universal plant primers (1) in a final volume of 20µl are shown. A ΔCq of 3.3 indicates no inhibitor presence (red line). N=3.

Reference:

1. Wang, J. *et al.* (2011) Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* **7**, 39.