Promega

Product Application

DNA Purification from Strawberry Leaves using the Maxwell® RSC System

Isolate high-quality, amplifiable DNA from strawberry leaf tissue using the Maxwell® RSC System.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: NanoDrop® absorbance, QuantiFluor® dsDNA, agarose gel electrophoresis, qPCR

Sample Type(s): *Fragaria ananassa* (strawberry leaf)

Input: 50mg and 100mg

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 TM473, available at: www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Zymo-Spin™ IV-HRC Columns (Zymo Cat.# C1010-50)
- 2.0ml Screw Cap Tubes (MP Cat.# 5076-400)
- Homogenization Steal Bead (MP Cat.# 2150)
- bead-beating device (MP Biomedicals Fast-Prep®-24 Instrument)
- microcentrifuge

Protocol:

1. Weigh 100mg of strawberry plant leaf, and place into a 2ml screw-top tube.
2. Add 1ml of CTAB Buffer to each tube.
3. Add 20µl of RNase A Solution and 40µl of Proteinase K Solution to each tube.
4. Using the bead-beating device, homogenize samples for desired time (e.g., FastPrep-Instrument at 4M/S, 20 seconds × 4, with a 20-second delay between each cycle).
5. Tap, invert and vigorously vortex tubes until the sample is resuspended.
6. Incubate sample at 65°C for 90 minutes with shaking at 600rpm.
7. Prepare the Maxwell® cartridges by adding the plungers to Well #8 and adding 100µl of Elution Buffer to the bottom of each elution tube.
8. After incubation, invert or vortex tubes containing lysate to mix thoroughly.
9. Place the tubes with lysate into a microcentrifuge and spin at ≥16,000 × *g* for 10 minutes at room temperature to separate any solids and oils.
10. Add 300µl of Lysis Buffer to Well #1 of the reagent cartridge.
11. Transfer 300µl of cleared lysate sample to well #1 of the reagent cartridge. Avoid pipetting any solid material from the bottom of the tube or the surface of the liquid.
12. Run the Purefood GMO and Authentication method on the Maxwell® RSC Instrument.
13. After the Maxwell® method is complete, add the eluate to a prepared Zymo-Spin™ IV-HRC Column.
14. Spin at 8,000 x *g* for 1 minute. Discard the spin column.

Results:

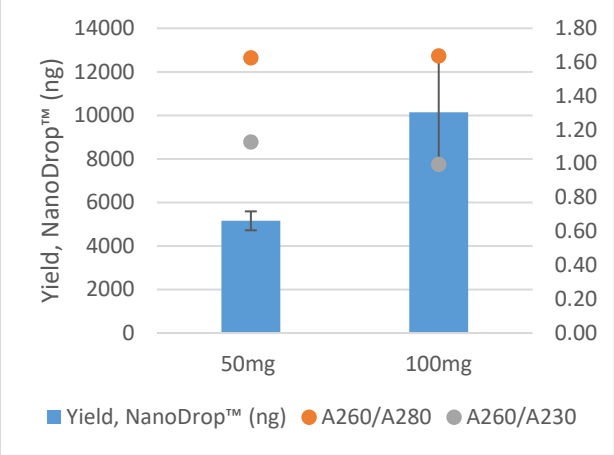

Figure 1. DNA yield and purity determined by absorbance ratios (NanoDrop® spectrophotometer). Data represents the mean ± SD (n=4) for each condition.

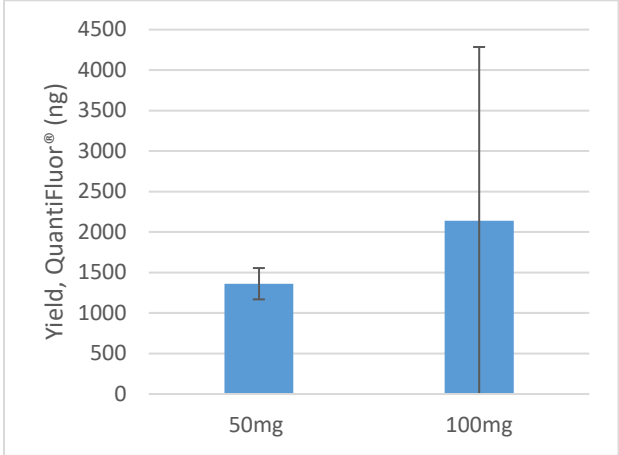

Figure 2. DNA yield measured by fluorescent dsDNA binding dye (QuantiFluor® ONE dsDNA System). Data represents the mean ± SD (n=4) for each condition.

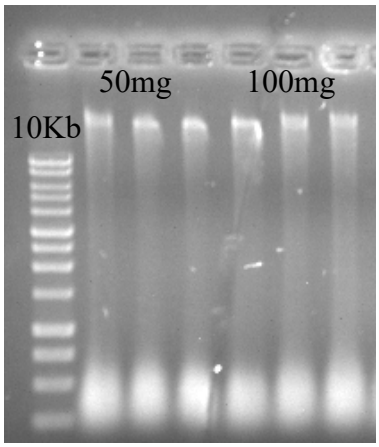

Figure 3. DNA integrity of purified strawberry DNA as determined by gel electrophoresis. DNA was purified from the indicated mass of strawberry leaf and analyzed by electrophoresis on a 1% agarose gel to assess integrity.

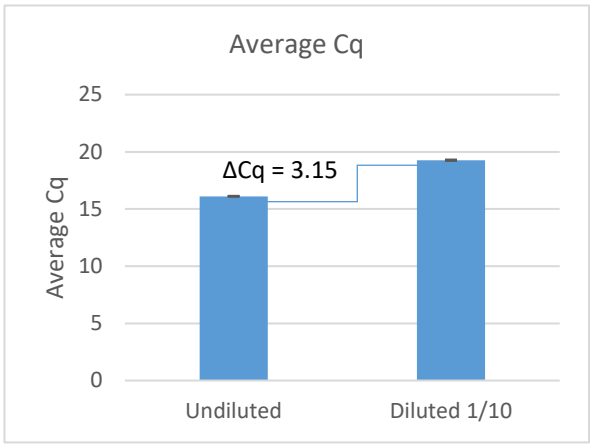

Figure 4. Inhibition of qPCR amplification in pooled strawberry DNA eluates. Undiluted or 1/10 diluted samples were used at a final volume of 10% in a qPCR assay. Assuming no amplification inhibition and 100% efficiency, a ΔCq of ~3.3 is expected between the undiluted and 1/10 diluted amplification reactions. Based on this analysis, the purified strawberry leaf DNA showed no amplification inhibition.