Promega

Product Application

Automated DNA Extraction from Grapes

Purify DNA pulp and skin samples from red and white grapes using the Maxwell® RSC Instrument and the Maxwell® RSC PureFood GMO and Authentication Kit.

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

Analyses: Dye-based quantification, qPCR

Sample Type(s): Red or white grapes from *Vitis vinifera* species

Input: 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 PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument (Cat.# AS4500)
- 1-thioglycerol (Cat.# A208B)
- Thermomixer (set at 65°C)
- Centrifuge
- PVP (polyvinylpyrrolidone) (Sigma)
- Bead beating device (e.g: FastPrep 24 from MP biomedical)
- Bead (¼" Ceramic sphere from MP biomedical)

Protocol:

1. Place 100mg of samples (pulp or skin) into a bead-beating tube with one ¼ ceramic bead.
2. Add 1ml of CTAB buffer with 40µl Proteinase K + 20µl RNase + 2% 1-thioglycerol + 2% PVP.
3. Grind the sample using a bead beating device twice at 5.5m/s for 30 seconds.
4. Incubate for 30min at 65°C at 600rpm.
5. Centrifuge samples for 5 minutes at 12,000 x *g* for 10min.
6. Prepare cartridges
    a. Place cartridges in RSC cartridge rack and remove foil seals.
    b. Add 50-100µl of Elution Buffer to Elution Tubes and place tubes in the cartridge rack.
    c. Place plungers into well #8 of the cartridge.
    d. Add 300µl Lysis Buffer into well #1 of the cartridge.
7. Add 300µl of supernatant of well #1 of the cartridge.
8. Run Maxwell® RSC PureFood GMO and Authentication Protocol.

Results:

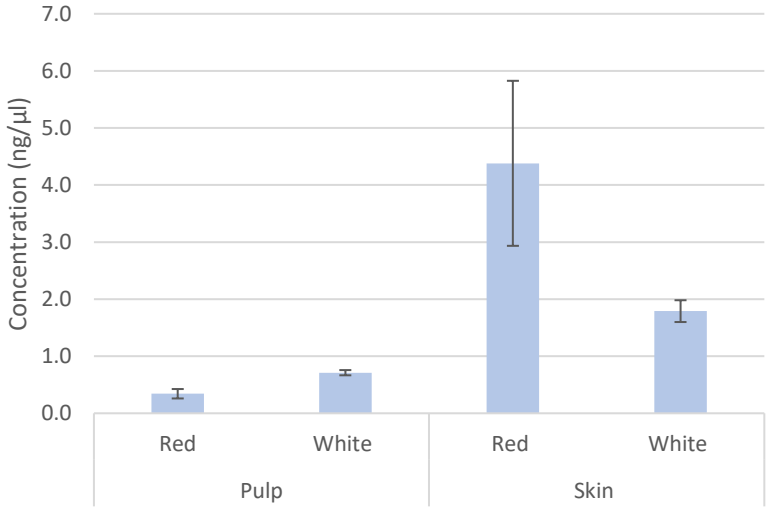

Figure 1: Concentration of DNA extracted from 100mg of grape skin or pulp using Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600).
Samples were eluted in 50µl. Quantitation was performed using a QuantiFluor® ONE dsDNA System (Cat.# E4870). Data are shown as mean ± STD of n=3.

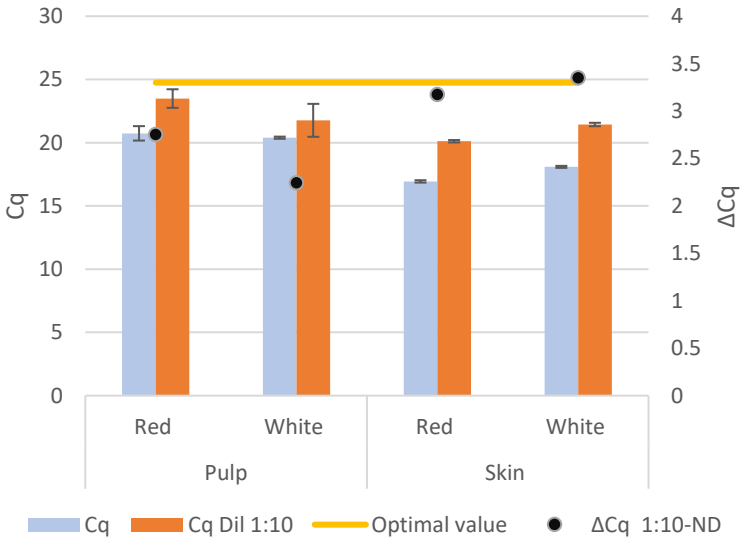

Figure 2: Cq and ΔCq analysis of DNA extracted from 100mg of grape skin or pulp. Cq and ΔCq values for 2µl of no dilution and 1:10 dilution of the extracted DNA amplified using GoTaq® qPCR Master Mix (Cat.# A6002) and Universal plant primers[1] in a final volume of 20µl. A ΔCq of 3.3 indicates no inhibitors present. N=3.

References:

1. Wang et al. 2011, Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* 7:3.