Promega

Product Application

Automated purification of DNA from microorganisms in wine

Purify DNA from microorganisms present in non-filtered wine samples 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: UV absorbance and qPCR

Sample Type(s): non-filtered wine

Input: 2ml

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 **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)
- Heat block capable of shaking (e.g. ThermoMixer® C from Eppendorf)
- Centrifuge
- PVP (polyvinylpyrrolidone)
- 1-Thioglycerol
- Bead beating device (e.g. FastPrep-24™ from MP Biomedicals)
- Lysing matrix (e.g. Lysing Matrix E from MP Biomedicals)

Protocol:

1. Centrifuge up to 2ml of wine at max speed for 10 minutes. Discard supernatant.
2. Resuspend the pellet in a solution of 1ml CTAB buffer, 40µl Proteinase K, 20µl RNase A Solution, 20µl 1-Thioglycerol, and 2% PVP.
3. Grind the sample using a bead beating device at 5.5m/s for 30 seconds.
4. Repeat step 3.
5. Incubate for 30 minutes at 65°C, shaking at 600rpm.
6. Centrifuge samples for 5 minutes at 12,000 x *g*.
7. Prepare cartridges
    a. Place cartridges into the RSC cartridge rack and remove foil seals.
    b. Add 50-100µl of Elution Buffer into each Elution Tube and place tubes into the cartridge rack.
    c. Place plungers into well #8 of the cartridges.
    d. Add 300µl of Lysis Buffer into well #1 of the cartridges.
8. Add 300µl of supernatant into well #1 of the cartridges.
9. Run the Maxwell® RSC with the PureFood GMO and Authentication Protocol.

Results:

Table 1. Concentrations and purity ratios of DNA extracted from 2ml of non-filtered wine samples collected from the bottom or top of the tank. DNA was eluted in 50µl. Eluates were analyzed spectrophotometrically using the NanoDrop™ One (Thermo Fisher Scientific). Data are shown as mean ± STD of n=3.

		ng/µl	A260/A280	A260/A230
Rosé wine	bottom of tank	56.0 ± 1.1	1.8	1.7
	top of tank	16.9 ± 2.5	1.5	1.0
White wine	bottom of tank	626.8 ± 105.2	2.1	2.2
	top of tank	18.7 ± 1.2	1.5	1.1

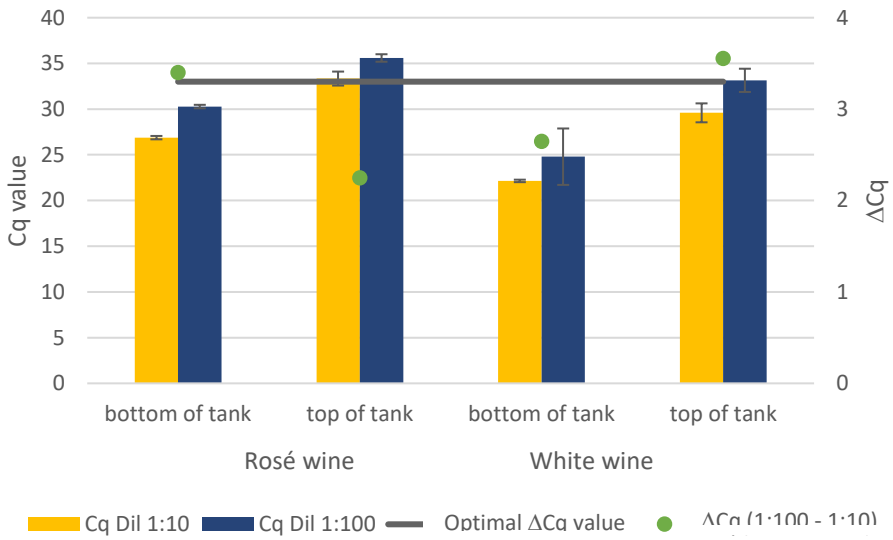

Figure 1. Cq and Delta Cq analysis of DNA extracted from 2ml samples of non-filtered wine using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). Cq values resulting from the amplification of 1:10 diluted and 1:100 diluted DNA eluates using GoTaq® qPCR Master Mix (Cat.# A6002) with S. *cerevisiae* specific primers are displayed. Delta Cq values (1:100-1:10) are also displayed. A ΔCq of 3.3 indicates no detectable inhibition. N=3.