Promega

Product Application

DNA Purification from Parsley Leaves using the Maxwell® RSC System

Isolation of amplifiable DNA from lyophilized parsley leaves using Maxwell® RSC Plant DNA Kit protocol.

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

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

Sample Type(s): Dried parsley (purchased)

Input: Equivalent of 5mg of dried parsley leaf per cartridge

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:

- FastPrep® Instrument (MP Biomedicals)
- Matrix D (MP Biomedicals, Cat.# 116913050) 1.4mm ceramic spheres
- Maxwell® RSC Instrument (Cat.# AS4500)
- Quantus™ Fluorometer (Cat.# E6150)
- NanoDrop® One Spectrophotometer (Thermo Fisher Scientific)
- GoTaq® qPCR Master Mix for dye-based detection (Cat.# A6002)
- Applied Biosystems® 7500 Real-Time PCR System

Protocol:

1. Prepare bead beating tube (Matrix D) with 20mg of dried parsley leaves, and add 600µl of TLA Buffer.
2. Bead beat 6m/s for 40 seconds on FastPrep® Instrument.
3. Centrifuge Vmax for 3 minutes in a tabletop microcentrifuge, and transfer lysate to a new 1.5ml tube.
4. Prepare cartridges as indicated in the Maxwell® RSC Plant DNA Kit Technical Manual TM458.
5. Load 150µl of lysate in duplicate in well #1 of Maxwell® RSC DNA Plant cartridges.
6. Add 150µl of Elution Buffer in Elution Tubes.
7. Start the purification procedure on the Maxwell® RSC Instrument.

Results:

Five milligrams (5mg) of dried parsley leaves were lysed and purified using the Maxwell® RSC Plant DNA Kit. Concentration of the eluted DNA was measured by absorbance-based (NanoDrop® One Spectrophotometer) and fluorescence-based (QuantiFluor® ONE dsDNA System) methods. DNA quality was assessed by absorbance purity ratios and qPCR inhibition. This protocol resulted in high yields of amplifiable DNA, though purity ratios were low and eluates were tinted.

Table 1. Analysis of DNA purified from 5mg of dried parsley leaves using the Maxwell® RSC DNA Plant Kit. After purification, the concentration of DNA eluates was measured by using absorbance NanoDrop® One Spectrophotometer and fluorescence (QuantiFluor® ONE dsDNA System) methods. Yields were calculated for 150µl elution volume based on quantification with the QuantiFluor® System. Purity ratios were measured using a NanoDrop® One Spectrophotometer.

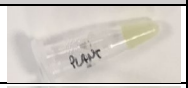
Maxwell Kit	Eluate	Picture of Eluates	NanoDrop® One			QuantiFluor® ONE	
			Concentration (ng/µl)	A_{260}/A_{280}	A_{260}/A_{230}	Concentration (ng/µl)	Yield (µg)
Maxwell® DNA Plant Kit	#1		542.63	1.55	0.60	25	3.75
	#2		532.77	1.53	0.61	23	3.45

Table 2. qPCR analysis of DNA eluates purified from 5mg of dried parsley leaves. After purification, eluates were diluted 1:10 and 1:100, and 2µl was amplified with GoTaq® qPCR System with plant-specific primers (1). Average Cq values were calculated for each condition from qPCR technical replicates. ΔCq were calculated between Cq values obtained from undiluted eluates and 1:10 dilutions, and between Cq values obtained from 1:10 and 1:100 dilutions. Shown is the average ± standard deviation for N=2 or 3 technical replicates. Theoretical value of 3.3 represents amplification without inhibition.

Eluate	Technical Replication	Mean Cq Value Undiluted	Mean Cq Value 1:10 dilution	Mean Cq Value 1:100 dilution	ΔCq ($Cq_{1:10}$-$Cq_{undiluted}$)	ΔCq ($Cq_{1:100}$-$Cq_{1:10}$)
#1	N=3	14.12 ± 0.23	16.80 ± 0.14	20.04 ± 0.10	2.67 ± 0.11	3.25 ± 0.18
#2	N=2	14.39 ± 0.08	16.95 ± 0.09	19.86 ± 0.07	2.56 ± 0.01	2.91 ± 0.02

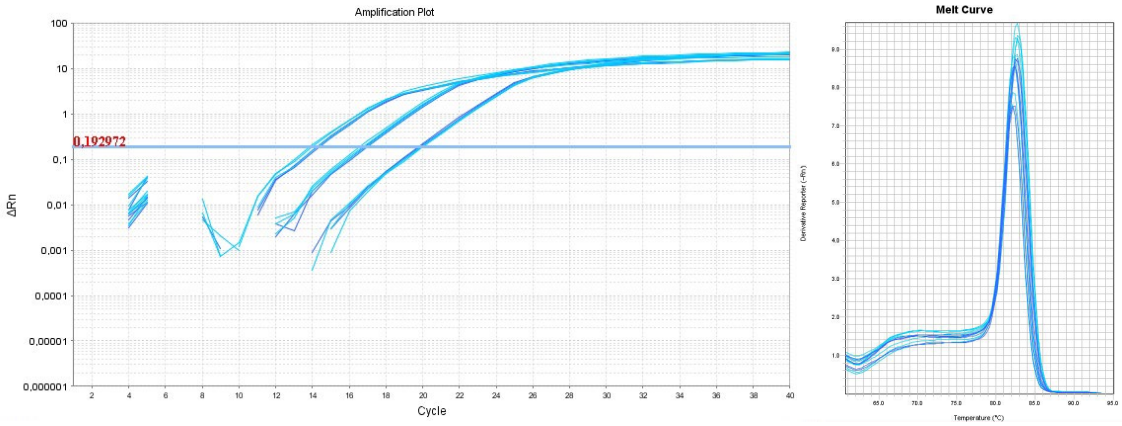


Figure 1. Semi-log plot and and melt curve of the qPCR amplification from purified DNA. Semi-log plot of the qPCR amplification (left) and melt curve (right) obtained on Applied Biosystems® 7500 Real-Time PCR System. Turquoise and blue curves correspond to amplification of eluates #1 and #2, respectively. The qPCR reaction efficiency was 112% with an R^2 value of 0.997 and a slope value of –3.055.

Reference:

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