Promega

Product Application

DNA Extraction from Herb and Spice Samples using the Maxwell® RSC System

Genomic DNA extraction from herb and spice samples using the Maxwell® RSC PureFood GMO and Authentication Kit for speciation/authentication.

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

Analyses: Quantitation by absorbance and fluorescence dye, qPCR amplification

Sample Type(s): Herbs: thyme, rosemary, coriander, dill, basil, parsley
Spices: black pepper, paprika, cumin, cinnamon, curry

Input: 1 gram of ground herb or spice

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, please contact Technical Services at: **techserv@promega.com**

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- NanoDrop® One Spectrophotometer
- QuantiFluor®ONE dsDNA System (Cat.# E4870)
- GloMax® Discover System (Cat.# GM3000)
- GoTaq® qPCR Master Mix (Cat.# A6002)
- for optional cleanup step: ProNex® Size-Selective DNA Purification System (Cat.# NG2001)

Protocol:

1. Add 10ml of CTAB buffer, 200µl of RNase A and 400µl of Proteinase K (PK) to each tube containing 1g of ground herb or spice.
2. Tap, invert and vigorously vortex tubes until the sample is suspended.
3. Place samples in a water bath at 65°C for 30 minutes. Shake the tubes every 5 minutes during the incubation.
4. Prepare RSC cartridges as indicated in Technical Manual TM473.
5. After incubation, invert or vortex tubes with lysate to mix thoroughly.
6. Place the tubes with lysate into a centrifuge and centrifuge at room temperature for 10 minutes at 12,000 × *g* to separate any solids and oils.
7. Transfer as much of the supernatant as possible to a clean 1.5ml microtube, avoiding the transfer of any solid and oils. Place the tubes into a microcentrifuge and spin at room temperature for 10 minutes at 13,400rpm.
8. Transfer 300µl of cleared lysate sample into the well #1 of the reagent cartridge. Avoid pipetting any solid material from the bottom of the tube or oil from the surface.
9. Place the cartridges in the deck tray, and run the Maxwell® RSC PureFood GMO and Authentication protocol.

Optional cleanup step for eluates containing inhibitor compounds (colored eluates or samples showing qPCR inhibition). Use the ProNex® Size-Selective DNA Purification System according to the protocol from the Technical Manual (TM508) with 3X (v/v) ratio of ProNex® Chemistry to eluate volume.

Results:

Table 1. DNA concentration and purity ratios obtained by extraction of DNA from herb and spice samples using the Maxwell® RSC PureFood GMO and Authentication Kit. One gram of sample was lysed on 10ml of CTAB buffer, and 300µl of lysate (corresponding to 28mg of sample) was used to perform the extraction using Maxwell® RSC Instrument. Sample absorbance was measured on the NanoDrop® One Spectrophotometer, and concentration was determined using the QuantiFluor® ONE dsDNA (Cat.# E4870). Standard deviation is shown (mean ± STD for n=3).

		NanoDrop® One dsDNA			QuantiFluor® ONE dsDNA (ng/µl)
Sample Type	**Sample**	**Concentration (ng/µl)**	**A260/280**	**A260/230**	**Concentration (ng/µl)**
Herbs	Thyme	202.9 ± 5.07	1.97	1.77	27.6 ±1.7
	Rosemary	74.1 ± 0.88	1.87	1.67	27.5 ± 1.54
	Coriander	514.7 ± 13.55	1.94	1.65	136.3 ± 42.49
	Dill	558.1 ± 26.57	2.06	2.23	178.4 ± 44.18
	Basil	607.7 ± 24.4	1.96	1.58	58.5 ±7.23
	Parsley	632.2 ± 22.77	1.98	2.05	144.9 ± 31.4
Spices	Black pepper	47.1 ± 1.03	1.46	0.77	1.8 ± 0.17
	Paprika	264.8 ± 6.67	1.70	1.16	26.9 ± 3.79
	Cumin	401.8 ±15.65	2.00	2.01	78.4 ± 11.31
	Cinnamon	36.7 ±2.89	1.45	0.72	0.6 ± 0.03
	Curry	212.7 ±4.01	1.81	1.38	32.8 ± 5.45

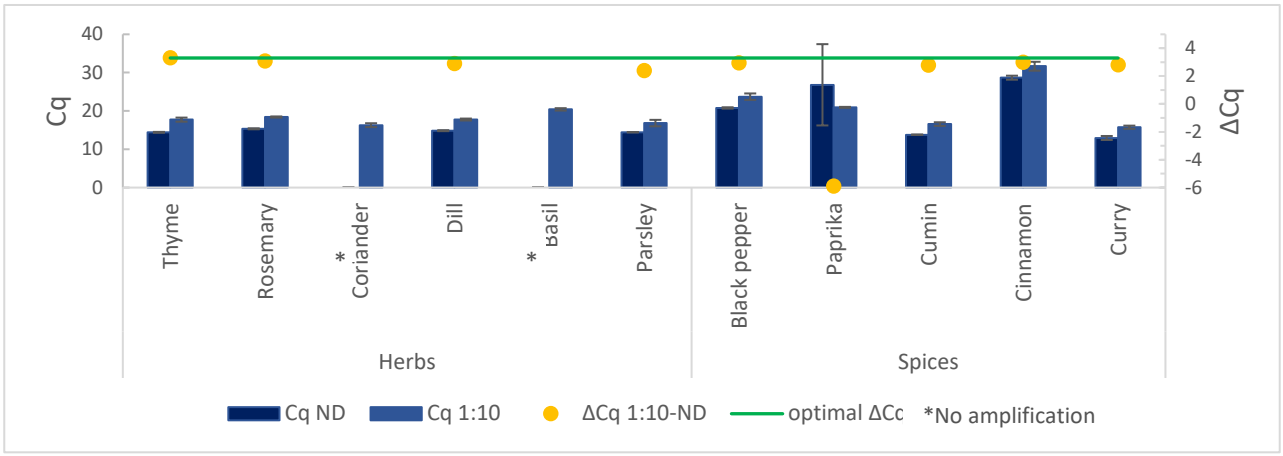

Figure 1. qPCR amplification results for DNA extracted from herb and spice samples using Maxwell® RSC PureFood GMO and Authentication Kit. One gram of sample was lysed on 10ml of CTAB buffer, and 300µl of lysate (corresponding to 28mg of sample) was used to perform the extraction using Maxwell® RSC Instrument. Cq and ΔCq values are the result of 2µl of undiluted and 1:10 dilution of the eluates amplified using GoTaq® qPCR Master Mix (Cat.# A6002) with universal plant primers (1) in a final volume of 20µl. The ΔCq between undiluted and 1:10 diluted sample is used as an indicator of the presence or absence of qPCR inhibitors. A ΔCq value of 3.3 for the 1:10 sample dilution relative to the undiluted sample mathematically represents 100% amplification efficiency and suggests no amplification inhibition. Standard deviation is shown (mean ± STD for n=3).

Optional Cleanup Step Results:

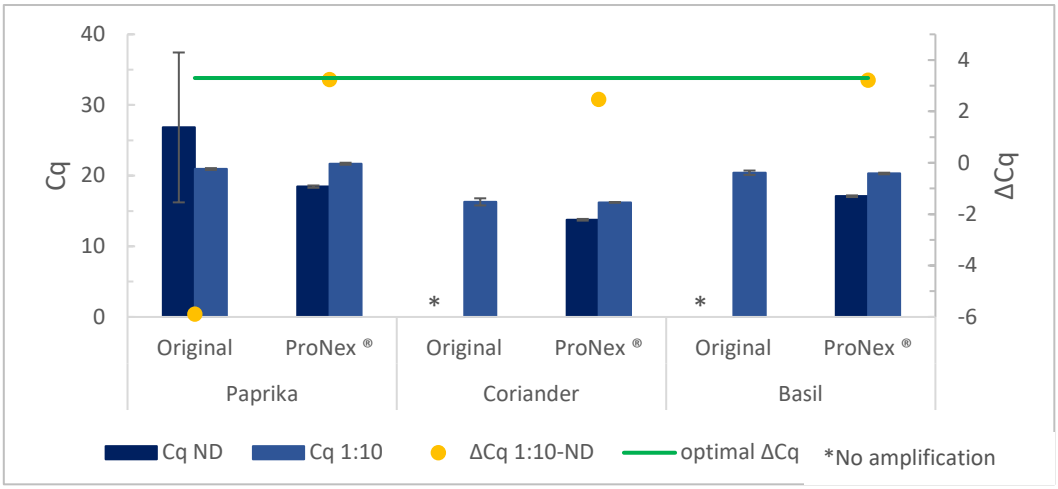

Figure 2. qPCR amplification results for DNA extracted from herb and spice samples with presence of inhibitor compounds before and after treatment with ProNex® Size-Selective DNA Purification System. Cq and ΔCq values are the result of 2µl of undiluted (ND) and 1:10 dilution of the eluates amplified using GoTaq® qPCR Master Mix (Cat.# A6002) with universal plant primers in a final volume of 20µl. The ΔCq between undiluted and 1:10 diluted sample is used as an indicator of the presence or absence of qPCR inhibitors. A ΔCq value of 3.3 for the 1:10 sample dilution relative to the undiluted sample mathematically represents 100% amplification efficiency and suggests no amplification inhibition. Standard deviation is shown (mean ± STD for n=3).

Reference:

1. Wang, J. *et al*. (2005) Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* **7**, 39.