Promega

Product Application

Automated Purification of DNA from Shrimp

Purify DNA from shrimp tissue using the Maxwell® RSC PureFood GMO and Authentication Kit on the Maxwell® RSC Instrument.

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

Analyses:

- Dye-based quantification
- Gel electrophoresis
- qPCR

Sample Type(s): Shrimp tissue stored at 4°C

Input: ≤100mg

Materials Required:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat block set to 65°C

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**

Protocol:

1. Cut and weigh 100mg of shrimp tissue on ice. Transfer to a 1.5ml tube.
2. Add 1ml of CTAB Buffer to each tube containing 100mg of sample.
3. Add 20µl of RNase A Solution and 40µl of Proteinase K Solution to each tube.
4. Mix sample thoroughly by inverting several times or vortexing.
5. Incubate in a heat block at 65°C for 30 minutes. Vortex 1-2 times during incubation.
6. During incubation, prepare RSC cartridges as described in the Maxwell RSC PureFood GMO and Authentication Kit Technical Manual (TM473):
    a. Add 300µl of Lysis Buffer to well #1 of each cartridge.
    b. Place a plunger in well #8 of each cartridge.
    c. Add 100µl of Elution Buffer to each Elution Tube.
7. After incubation, invert or vortex tubes with lysate to mix thoroughly.
8. Centrifuge tubes at room temperature for 10 minutes at 16,000 x *g*.
9. Transfer 300µl of cleared lysate to well #1 of each cartridge.
10. Select the Maxwell® RSC PureFood GMO and Authentication method and run.

Results:

DNA was successfully purified from 100mg of shrimp tissue stored at 4°C using the Maxwell® RSC PureFood GMO and Authentication Kit on the Maxwell® RSC Instrument. DNA was amplifiable in both endpoint and qPCR assays, though eluates needed to be diluted 1:10 in Nuclease-Free Water for endpoint amplification.

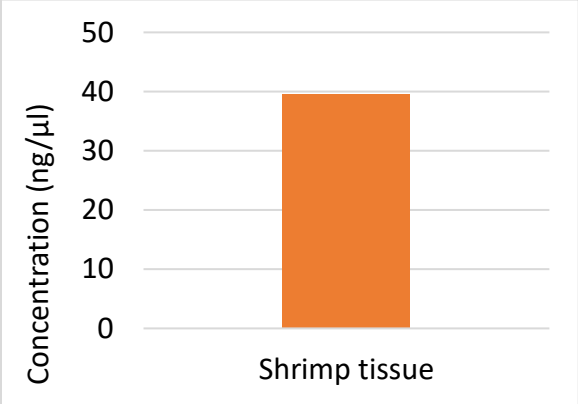

Figure 1. DNA eluate concentration results. DNA was purified from 100mg of shrimp tissue using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). The eluate DNA concentrations were measured using the QuantiFluor® ONE dsDNA System (Cat.# E4871) on the Quantus™ Fluorometer (Cat.# E6150). Data represent the average concentration of eluates from duplicate purifications

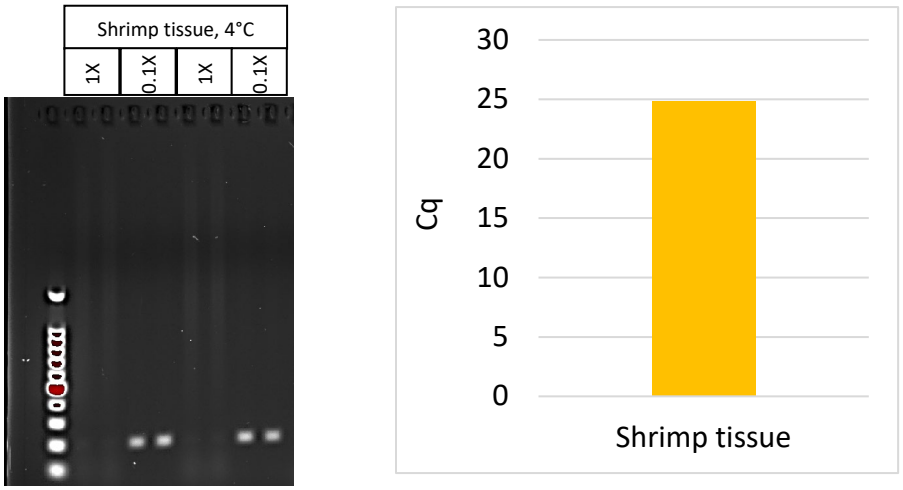

Figure 2. Amplification of purified DNA eluates. DNA was purified from 100mg of shrimp tissue using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). **Left: Endpoint PCR amplification results.** The eluates from duplicate purifications were amplified in duplicate with primers specific to a decapod mitochondrial 16S rRNA gene[1] using GoTaq G2 Hot Start Green Master Mix (Cat.# M7422). **Right: qPCR amplification results.** DNA eluates were amplified using qPCR primers specific to a mitochondrial 16S rRNA gene.[2] DNA was detected using GoTaq® qPCR System (Cat.# A6001). Data represent the average Cq value for a single amplification replicate of eluates from duplicate purifications.

References:

1. Komai, T., et al. (2019). Development of a new set of PCR primers for eDNA metabarcoding decapod crustaceans, *Metabarcoding and Metagenomics,* 3:1-19
2. Sawyer, J. et al. (2003). Real-time PCR for quantitative meat species testing, *Food Control*. **14**, 579-583.