Promega

Product Application

Automated Purification of DNA from Fish Stored in Ethanol

Purify DNA from fish tissue and fin stored in ethanol 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): Salmon tissue, bass tissue and fins stored at -20°C, 4°C, or in ethanol 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 fish tissue or fin 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 salmon and bass samples stored at -20°C, 4°C, or in ethanol 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.

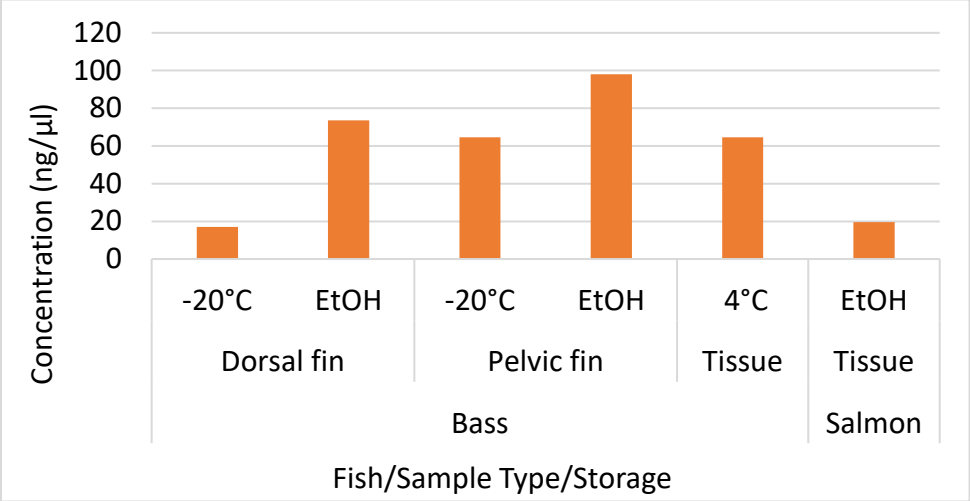

Figure 1. DNA eluate concentration results. DNA was purified from 100mg fish samples using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). Sample types included bass dorsal and pelvic fins stored at -20°C or in ethanol at 4°C (EtOH), bass tissue stored at 4°C, and salmon tissue stored in ethanol at 4°C. 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.

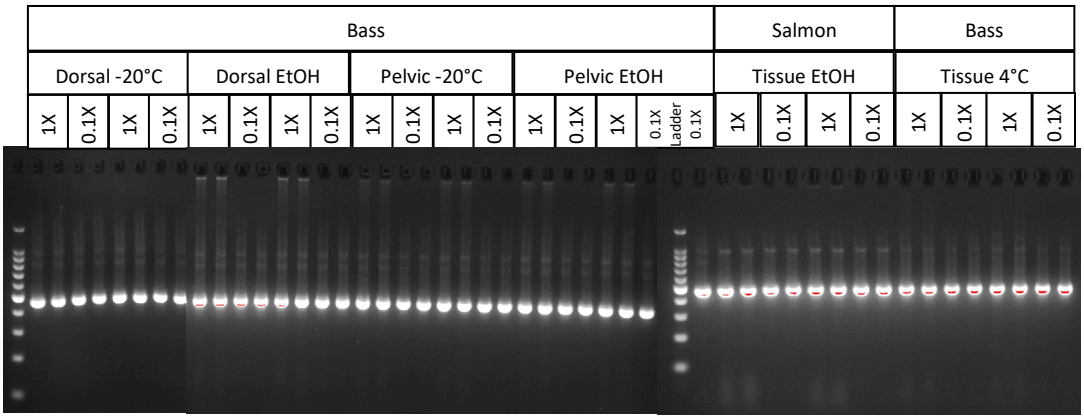

Figure 2. Endpoint PCR amplification results. DNA was purified from 100mg fish samples using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). Sample types included bass dorsal and pelvic fins stored at -20°C or in ethanol at 4°C (EtOH), bass tissue stored at 4°C, and salmon tissue stored in ethanol at 4°C. The eluates from duplicate purifications were amplified in duplicate with primers specific to the fish cytochrome b gene[1] using GoTaq® G2 Hot Start Green Master Mix (Cat.# M7422).

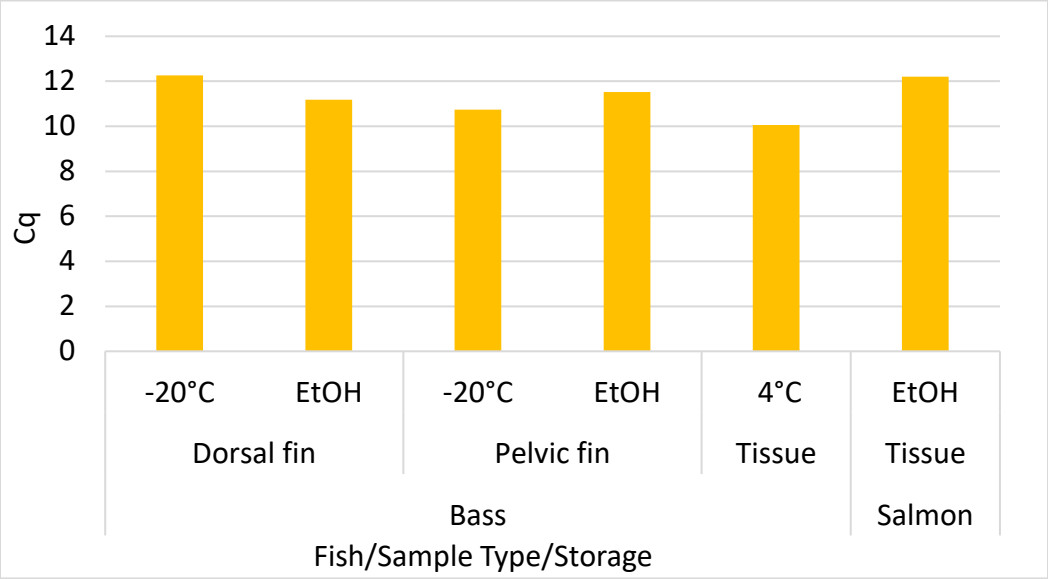

Figure 3. qPCR amplification results. DNA was purified from 100mg fish samples using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). Sample types included bass dorsal and pelvic fins stored at -20°C or in ethanol at 4°C (EtOH), bass tissue stored at 4°C, and salmon tissue stored in ethanol at 4°C. 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. Application Report: Maxwell DNA Extractions for Fish Samples. Alisha Truman and Brad Hook. 01/14.

2. Sawyer, J. et al. (2003). Real-time PCR for quantitative meat species testing, *Food Control*. **14**, 579-583.