Promega

Product Application

Automated purification of DNA from horse meat

Purify DNA from horse meat using Maxwell® RSC instrument and Maxwell® RSC Purefood GMO and Authentication kit

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

Analyses: UV-absorbance, TapeStation and qPCR

Sample Type(s): Horse meat

Input: 50 to 200mg of meat samples

Materials Required:

- Maxwell® RSC Purefood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heater/shaker for tubes (e.g. ThermoMixer C, Eppendorf)
- Microcentrifuge
- 1.5 or 2ml screw cap tubes

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 meat sample into small pieces using a scalpel.
2. Transfer to 1.5 or 2ml screw cap tubes.
3. Prepare a digestion mix (600µl of CTAB+2µl RNase A+30µl Proteinase K per sample).
4. Add 632µl of digestion mix in each tube.
5. Incubate 30min to 1h at 60°C under 1,600 rpm agitation.
6. Prepare cartridges by adding 300µl of Lysis Buffer in well #1 and 100µl Elution Buffer in Elution tubes.
7. After incubation, centrifuge meat lysates at 13,400 rpm for 10min.
8. Load 300µl of cleared lysates in well #1 of cartridges and tip mix.
9. Start Purefood GMO and Authentication procedure on Maxwell® RSC Instrument.

Results:

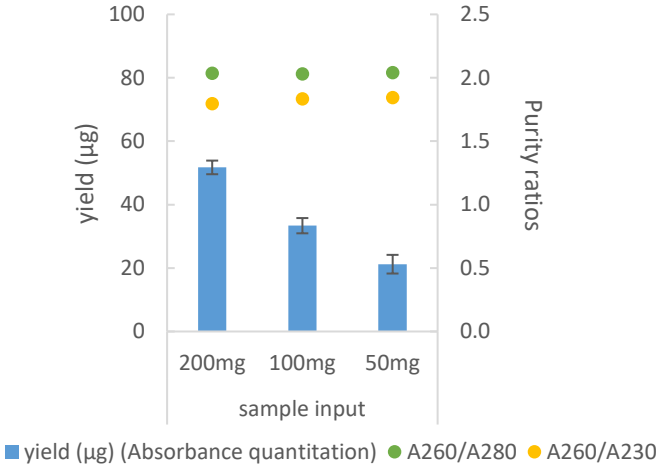

Figure 1: Yields and purity ratios of DNA purified from different inputs of beef and horse meats. 200mg, 100mg or 50mg of fresh meat were purified using Maxwell® RSC Purefood GMO and Authentication Kit (Cat.# AS1600). Purified DNA were analyzed using absorbance (Tecan Spark spectrophotometer with NanoQuant Plate). Average yields were calculated based on concentrations measured by absorbance for 100µl elution volume. Shown is the average ± standard deviation for N=3.

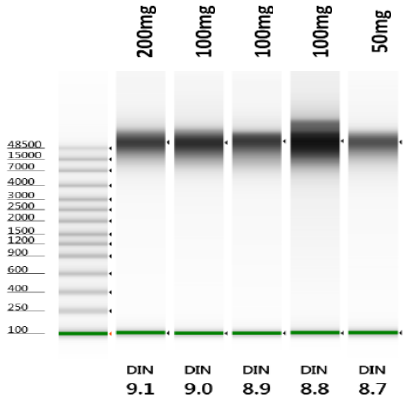

Figure 2: TapeStation migration of DNA purified from different inputs of beef and horse meats. 200mg, 100mg or 50mg of fresh meat were purified using Maxwell® RSC Purefood GMO and Authentication Kit (Cat.# AS1600). Purified DNA were analyzed using TapeStation instrument (Agilent) with a gDNA ScreenTape.

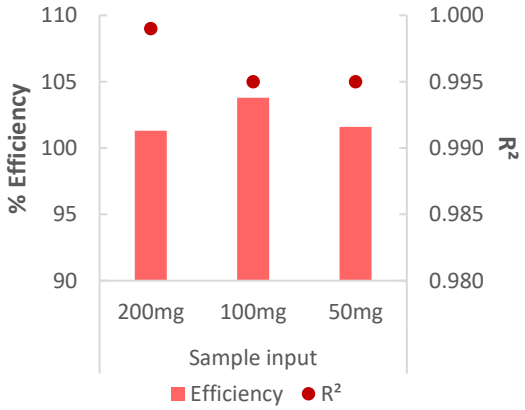

Figure 3: qPCR amplification efficiencies and R^2 of standard curves generated with DNA purified from beef and horse meats using a mammalian-specific probe[1]. Three eluates of each condition (biological replicates) were diluted to generate standard curves (5points, 10 folds). Standard curves were amplified using GoTaq® Probe qPCR Master Mix (Cat.# A6102) on a Bio-Rad CFX96™ instrument. All efficiencies and R^2 obtained from horse meat DNA were in the expected qPCR range. Inhibition would impact both R^2 and efficiencies. Average ΔCq obtained between undiluted eluates and 1/10 dilutions was on average 3.3±0.2 (N=3) compared to the theoretical value of 3.3.

Reference:

1. Laube, *et al.*, (2003) Methods for the detection of beef and pork in foods using real-time polymerase chain reaction, *International Journal of Food Science and Technology*. **38**, 111–118.