Promega

Product Application

Processed Grain DNA Purification

Isolate high quality, amplifiable DNA from processed grains using the Maxwell® 16 System.

Kit: Maxwell® 16 FFS Nucleic Acid Extraction Kit (Cat.# X9431)

Analyses: GoTaq® qPCR, QuantiFluor® quantitation

Sample Type(s): Tortillas, corn chips, biscuit, rice cake

Input: 50mg

This protocol was developed by Promega Applications Scientists and is intended for research use only.

User are responsible for determining suitability of the protocol for their application.

For further information, please contact **techserv@promega.com**

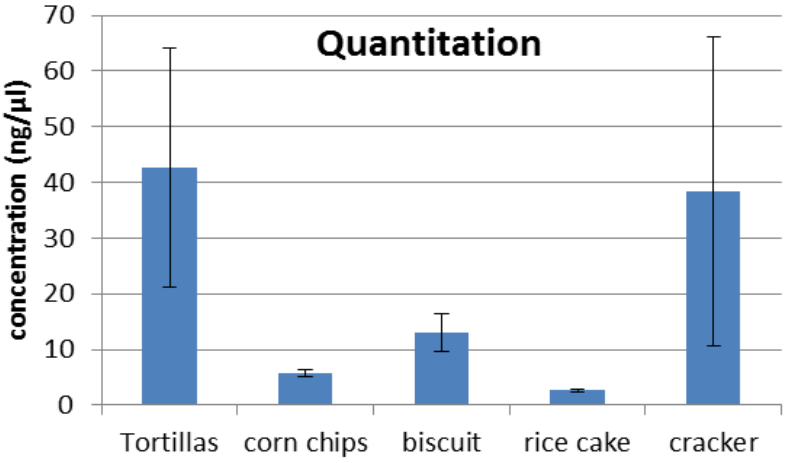
Materials Required:

- Maxwell® 16 Instrument (Cat.# AS2000) with firmware version 4.97
- Maxwell® 16 FFS Nucleic Acid Extraction Kit (custom Cat.# X9431)
- Optional: RNase A Solution (Cat.# A7973)
- CTAB Buffer: 2% CTAB, 1.4M NaCl, 0.1M Tris 10mM EDTA pH 8.0
- Optional: 1-Thioglycerol (Sigma, Cat.# M1753)
- Homogenization protocol only: Coffee grinder or other grinding apparatus
- Microcentrifuge
- Heat block to 65°C

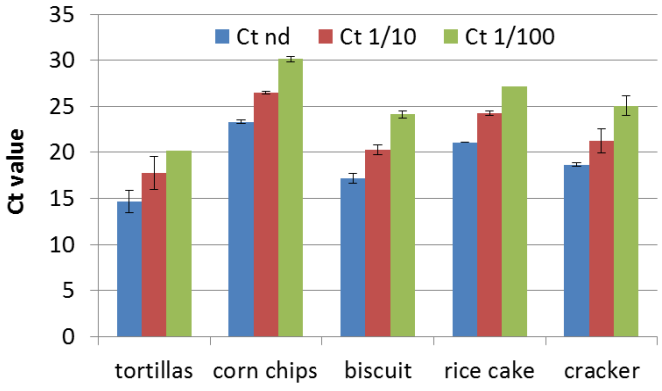
Protocol:

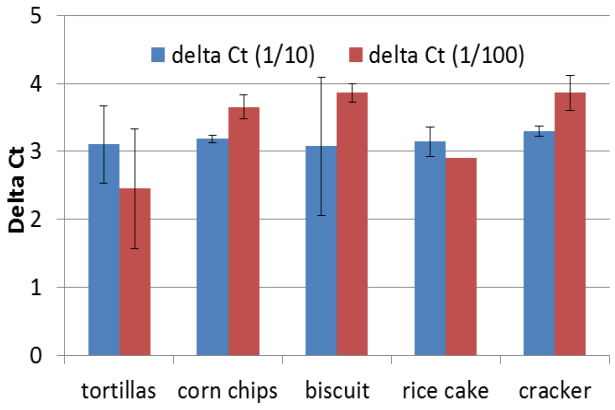
1. Cut sample in small pieces or grind (when possible) and place 50mg into a tube.
2. Add 600µl of fresh CTAB and 30µl Proteinase K.
3. Optional: Add 20µl RNase A solution.
4. Optional: In case of PCR inhibition, add 20µl thioglycerol to CTAB buffer.
5. Incubate for 90 minutes at 65°C.
6. Centrifuge for 10 minutes at high speed.
7. Pipet 300µl of sample and 300µl of Lysis Buffer into well #1 of the Maxwell® 16 Cartridge.
8. Place one of the supplied elution tubes into the sample rack and add 100µl of the supplied Elution Buffer for each sample.
9. Place the plunger in position in the cartridge.
10. Select LEV configuration on the Maxwell® Instrument and select method as follows: RUN, DNA: Plant. Start run.

Results



Concentration of purified DNA. DNA was extracted from 50mg of the indicated processed grains and eluted in 100µl. Concentration was determined using the Quantifluor® dsDNA System (Cat. # E2670). Values indicate the mean and standard deviation for n=3 samples.





Performance in qPCR. Processed grain DNA was amplified by qPCR using GoTaq® qPCR Master Mix (Cat.# A6001); n=3. **Left Panel:** Ct values of amplification reactions containing 1µl non-diluted (nd), 1/10 and 1/100 diluted DNA eluate. **Right Panel**: Delta Ct between serially diluted samples indicates no inhibition of PCR.