1. Abstract

The ability to trace and authenticate a food product is of major concern to the food industry. It has implications in consumer protection and regulatory compliance as well as cultural and religious aspects. Advances in molecular technologies and genomic analyses of food sources enable screening at the nucleic acid level. Here, we examine the utility of a small benchtop automated instrument, the Maxwell 16, and novel cellulose-based magnetic particles for purification of amplifiable DNA from a variety of foods. We choose 4 key areas of interest for food ingredient testing: genetically modified organisms (GMO) detection, seafood identification, seed screening (rice and coffee) and meat product testing. For each area, nucleic acid was extracted, quantitated, and tested for amplifiability in either end point or quantitative PCR. For GMO testing, extracted DNA from both dried kernels and processed foods (chips and pretzels) were tested for the presence of cauliflower mosaic virus (CMV) sequence. Results showed amplification of CMV sequences in GMO branded samples only. For seafood authentication, DNA was extracted from processed and frozen tuna and salmon and successfully amplified using endpoint PCR of cytochrome b, typically used for fish species identification. For seeds, DNA was purified and demonstrated to be amplifiable from rice (medium grain and basmati) and coffee beans (Arabica and Robusta). Meat testing focused on isolation of amplifiable nucleic acid from processed foods including ravioli, sausage, and gelatins. These studies together demonstrate the use of the Maxwell 16 System for automated purification of food DNA upstream of amplification-based GMO and authentication testing.

2. Maxwell® 16 Instrument for DNA extraction from a variety of foods

Food, Feed or Seed DNA:
1. Maxwell 16 FFS Kit, Custom (cat. #X9431) + CTAB Buffer (2% CTAB, 1.4M NaCl, 0.1M Tris, 10mM EDTA pH 8.0) + Proteinase K [RNase A Solution (cat. #A7973), optional]
2. Maxwell 16 FFS Kit, Custom (cat. #X9431) + TLA Buffer (cat. # A5091) + Proteinase K

3. Seafood Identification

Fish-DNA extraction

GoTaq® amplification of cytochrome b for PCR-RFLP analysis

Different meat and gelatin based products (50mg) were ground and DNA was extracted using CTAB Buffer as described. Concentrations were determined using QuantFluor® dsDNA System and a NanoDrop spectrophotometer. DNA was amplified using GoTaq® Green PCR Master Mix and primers specific for the cytochrome b gene (1) which is used for RFLP or sequencing analysis to identify fish species.

4. Purify Amplifiable Coffee Bean DNA

Arabica and Robusta coffee beans (100-200mg) were ground and DNA was extracted as described using CTAB. Yields were determined using QuantFluor® ONE dsDNA System. Samples were analyzed by real-time PCR using GoTaq® qPCR Master Mix and universal plant primers (2). The difference in Ct values between serially diluted samples indicate no inhibitors co-purified with the DNA.

5. Speciation of Single Rice Kernels

One rice kernel (without grinding) was directly added to the CTAB Buffer, and DNA was extracted as described. Concentrations were determined using QuantFluor® dsDNA System. DNA was examined by gel electrophoresis (0.5% agarose gel). DNA was amplified using the Rice Multiple-X™ PCR Kit (SoGenCo., Ltd.) and PCR products were visualized on 3% agarose gel.

6. Screen Meat Products for Animal Source

7. Detect GMO in Food

Corn Chips and Pretzel samples (50mg) were ground and DNA was extracted as described using CTAB buffer. Concentrations were determined using QuantFluor® dsDNA System. GMO detection was tested using GoTaq® qPCR Master Mix and primers specific to CMV 35S promoter (4).

8. GMO Screen Corn Kernels

Popcorn kernels (150mg) were ground or processed whole and DNA was extracted as described using CTAB buffer. DNA concentrations were determined using QuantFluor® ONE dsDNA System. DNA was amplified using GoTaq™ Green PCR Master Mix and primers for control gene (invertebrate) or GMO sequence (CryAb(b)↓). Lanes are marked as follows (↓) no template control reaction, (↓) commercial corn genomic DNA, (G) ground Generic corn DNA, (HG) ground non-GMO corn DNA, (1K) single whole kernel generic corn DNA.

9. Summary

The Maxwell® 16 system is an automated solution for purification of DNA from a variety of foods. We successfully extracted amplifiable DNA from:
- Salmon (fresh, pouch and canned), Tuna (pouch and can), and Cod (fresh) for seafood identification
- Coffee beans for detection of arabica and robusta beans
- Single rice kernels for speciation testing
- Processed food such as aspic, sausage, ravioli or soft gel capsule for authentication of meat species
- Processed food (corn chips and pretzel) for GMO detection, and
- Corn kernels (both ground or whole) for GMO detection

References:
2. Wang et al. (2011) Plant Methods, 7, 39.