Promega

Product Application

Coffee (*Coffea arabica* and *robusta*) Bean DNA Purification

Isolate high quality, amplifiable DNA from coffee beans using the Maxwell® 16 System.

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

Analyses: GoTaq® qPCR, QuantiFluor® quantitation, gel

Sample Type(s): *Coffea arabica* and *robusta* beans (un-roasted)

Input: Up to 200mg ground beans

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, please contact **techserv@promega.com**

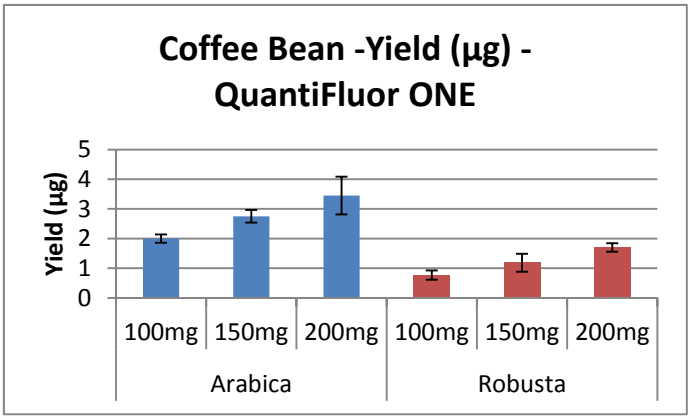
Materials Required:

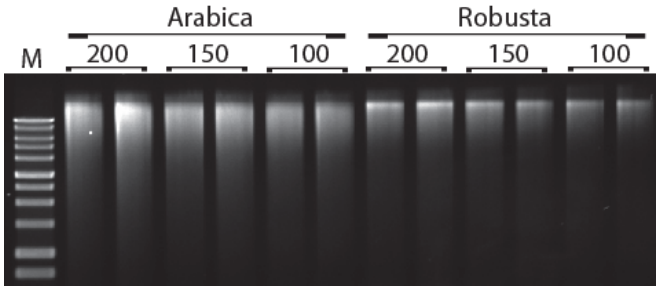
- Maxwell® 16 Instrument (Cat. # AS2000) with firmware version 4.97 or later
- Maxwell® 16 FFS Nucleic Acid Extraction Kit (custom Cat. #X9431)
- Optional: RNase A Solution (Cat. # A7973)
- 2.0mL screw-top tubes
- CTAB Buffer: 2% CTAB, 1.4M NaCl, 0.1M Tris 10mM EDTA pH 8.0
- Liquid nitrogen
- Mortar and pestle
- Microcentrifuge
- Heat block

Protocol:

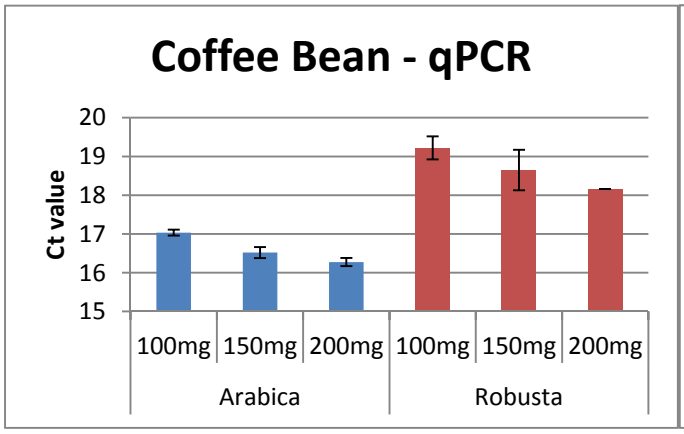
1. Grind beans in liquid nitrogen with a mortar and pestle.
2. Weigh up to 200mg of ground bean into a 2ml tube.
3. Add 1ml CTAB buffer spiked with 40µl Proteinase K and optional 20µl RNase A Solution.
4. Vortex vigorously then incubate 90 minutes at 65°C.
5. Centrifuge for 10 minutes at 16,000 *x g*.
6. Transfer 300µl sample supernatant and 300µl Lysis Buffer into well #1 of the Maxwell® 16 cartridge.
7. Place Elution Tubes into the sample rack and add 100µl of the supplied Elution Buffer for each sample.
8. Place the plunger in the indicated position of the cartridge.
9. Select LEV configuration on the Maxwell® Instrument and select the purification method as follows: RUN, DNA: Plant. Start run.

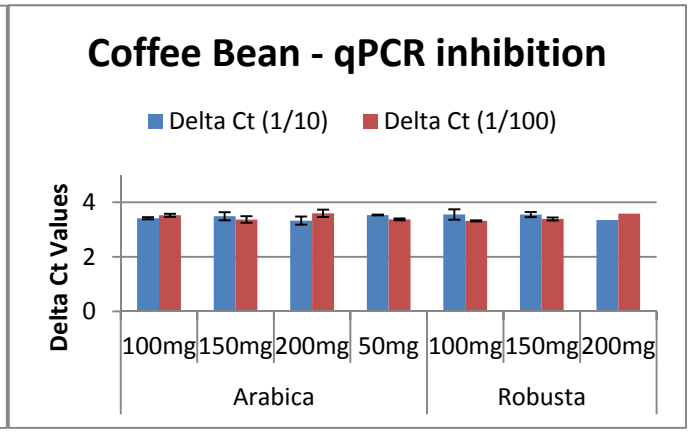
Results





DNA yield and quality. **Left Panel:** DNA yield from Arabica and Robusta coffee beans was determined by quantitation using the QuantiFluor® ONE dsDNA System (Cat. # E4871). **Right Panel:** Examples of high molecular weight DNA purified from coffee beans. 5µl DNA was run on a 1% agarose gel for 50 minutes at 70V. M = 6µl of BenchTop 1kb ladder (Cat. # G7541).





Peformance of extracted DNA in qPCR: Left Panel: Purified DNA samples were analyzed by real-time PCR using the GoTaq® qPCR Master Mix (Cat.# A6001), universal plant primers (1) and 1µl DNA in 25µl reactions. **Right Panel:** ΔCt of serially diluted samples indicates no significant qPCR inhibition.

Reference

1. Wang, J., *et al.,* (2011). Universal endogenous gene controls for bisulfite conversion in analysis of plant DNA methylation. *Plant Methods*. **7**, 39.