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

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.
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).

**Performance 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**