

### Cacao Plant Tissue DNA Purification

*Isolate high molecular weight, amplifiable DNA from cacao plant tissue using the Maxwell® 16 System.*

**Kit:** Maxwell® 16 LEV Plant DNA Kit  
(Cat. #AS1420)

**Analyses:** GoTaq® qPCR, QuantiFluor® quantitation, gel

**Sample Type(s):**

- Cacao leaf tissue, up to 150mg
- Cacao seed, up to 100mg
- Cacao root tissue, up to 150mg
- Cacao meristem tissue, 20mg

**Input:** CTAB sample supernatant, up to 300µl

**Materials Required:**

- Maxwell® 16 instrument (Cat. #AS2000) with firmware version 4.97 or later
- Maxwell® 16 LEV Plant DNA Kit (Cat.# AS1420)
- CTAB buffer: 2% CTAB, 1.4 M NaCl, 0.1M Tris, 20 mM EDTA pH 8.0
- RNase A (Cat. # A7973) (optional)
- Zymo-Spin™ IV-HRC columns (Zymo Research, Cat. # C1010-50)
- Liquid Nitrogen
- Mortar and Pestle
- 65°C Incubator
- Microcentrifuge

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.

Further information can be found in Technical Manual #TM414, available at: [www.promega.com/protocols](http://www.promega.com/protocols)

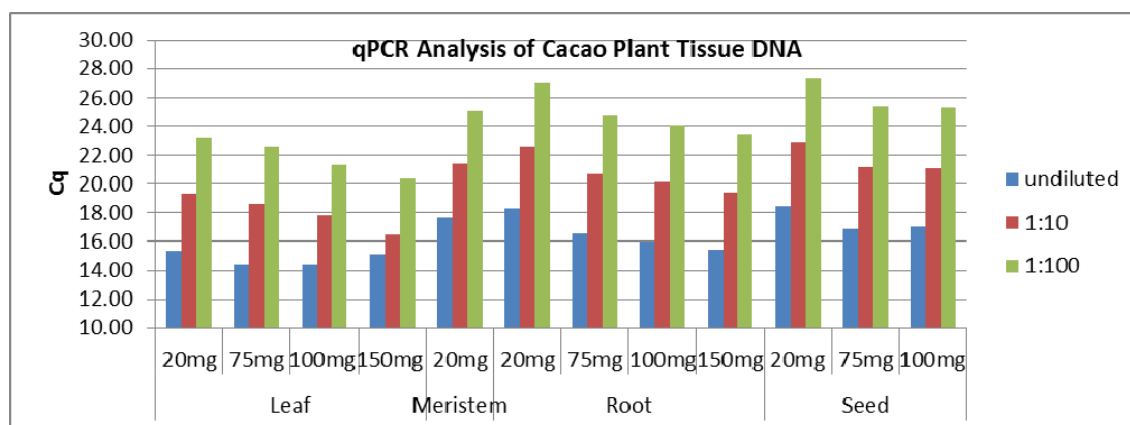
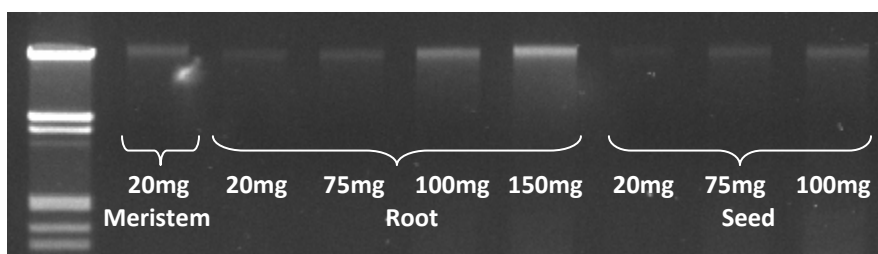
#### Protocol

1. Grind tissue sample material in liquid nitrogen using a mortar and pestle.
2. Add up to 150mg of the ground sample to a 2ml tube.
3. Add 1ml CTAB buffer, 40µl Proteinase K (20 mg/ml) and 20µl RNase A Solution (optional).
4. Vortex vigorously and incubate for 90 minutes at 65°C.
5. Centrifuge for 10 minutes at 16,000 x g (optional: centrifuge supernatant again).
6. Place the cartridge to be used into the Maxwell® LEV cartridge rack and remove the seal.
7. Add 300µl of cleared sample supernatant and 300µl of Lysis Buffer into well #1 of the Maxwell® cartridge.
8. Place Elution Tubes into the sample rack and add 100-150µl Elution Buffer for each sample.
9. Place the plunger in the indicated position of the cartridge.
10. Select LEV configuration on the Maxwell® Instrument and select the purification method as follows:  
RUN, DNA: Plant. Start run.

## Results

Plant Tissue	Mass	Purity		Concentration (ng/μl)	Yield (μg)
		A <sub>260</sub> /A <sub>280</sub>	A <sub>260</sub> /A <sub>230</sub>		
Meristem	20mg	1.97	1.19	1.55	2.17
Root	20mg	1.72	1.49	0.73	1.02
	75mg	1.96	1.80	1.79	2.42
	100mg	2.03	1.99	2.79	3.79
	150mg	2.00	1.98	4.42	6.14
Seed	20mg	1.60	1.02	0.67	0.88
	75mg	1.91	1.31	1.54	2.20
	100mg	1.91	1.58	1.95	2.79
Leaf	20mg	1.12	0.54	4.1	0.26
	75mg	1.30	0.62	8.2	0.42
	100mg	1.44	0.75	19.0	0.51
	150mg	1.46	0.78	29.0	0.64

**DNA purity, concentration and yield.** Concentration and yield were determined by using the QuantiFluor™ One dsDNA System (Cat. # E4871). Purity was determined using the NanoDrop®-1000 spectrophotometer.



**Quality and performance of extracted DNA: Top Panel:** High molecular weight DNA purified from various quantities of cacao plant meristem, root and seed tissue. The largest band of the DNA Marker (left lane) is 21,226bp. **Bottom Panel:** Analysis of purified DNA using GoTaq® qPCR Master Mix (Cat. # E6002). ΔC<sub>q</sub> of samples indicate little to no inhibition of serially diluted eluates. Slight inhibition was observed with DNA purified from 150mg of leaf tissue.