Genomic Extraction Protocol for QIAGEN Maxi (P-500) Columns*

1) Crush and grind fresh plant tissue in liquid N_2 with silica sand using a mortar and pestle. Use the appropriate amount of tissue for your crop and quality of tissue collected (large quantities of tissue may require increased volumes beginning in step 2, ie. >20 ml Extraction Buffer). Plant tissue should be store at -80° C prior to use. **Do not allow tissue to thaw** anytime prior to grinding. If using freeze-dried plant tissue, grind prior to step 2.

2) Add crushed samples to 50 ml conical, screw cap Falcon tubes. Add 20 ml (or appropriate volume) of sterile extraction buffer (50 mM Tris (pH 8.0), 0.7 M NaCl, 10 mM EDTA, 1% CTAB (hexadecyltrimethylammonium bromide), 0.25% b-mercaptoethanol, 0.025 mg/ml RNAase). **Use 2x stock solution for fresh tisse.** Use 1x stock solution for freezed-dried tissue.

To Make: 1 Liter, 2x Stock Extraction Buffer

1 M Tris (pH 8.0)	100 ml
NaCl	81.8 g
0.5 M EDTA	40 ml
CTAB	20 g
Heat to dissolve, Autoclave	

*Upon use add b-mercaptoethanol and RNAase. 50 ml b-mercaptoethanol to 20 ml extraction buffer, 50 ml RNAase (10 mg/ml) to 20 ml extraction buffer

3) Mix tissue in buffer until thoroughly wet. Final product should not be highly viscous.

4) Incubate at 50° C for 45 minutes, mix gently (so as not to break DNA) about every 10 minutes.

5) Add equal volume of chloroform:isoamyl alcohol (24:1) and incubate for 45 minutes at 50° C. Mix gently about every 10 minues.

6) Centrifuge in 50 ml tubes in SS34 rotors (use rubber sleeves) for 10 minutes at 8000 rpm, or 250 ml tubes for 10 minutes at 6000 rpm.

7) Using inverted 10 ml pipette (to provide large orifice), remove supernatant and place in a new acid-washed tube. Remove only the top phase.

8) Add an equal volume of isopropanol, or twice the volume or 95% ethanol. Invert gently until two phases are no longer evident. DNA will collect as white stringy mass. Sample may be stored overnight at -20° C at this point.

9) Centrifuge samples for 20 minutes to pellet DNA/contaminants.

10) Resuspend pellet in 12.5 ml 1 M NaCl. Allow DNA to redissolve at 62° C for ~30 minutes.

11) Add 4.25 ml dH20 and 8.25 mls QIAGEN Buffer QBT. Final volume 25 mls.

12) Equilibrate QIAGEN tip-500 Column with 10 ml QBT.

13) Vortex DNA sample 5-10 sec. Apply to column.

14) Wash column with 20 ml QC Buffer. **Wash 3 times.**

15) Elute DNA with 15 mls QF into silanized Corex tubes.

16) Add 10.5 mls isopropanol, mix gently (DNA appears as fine threads).

17) Centrifuge for 20 minutes. Pour off supernatant.

18) Wash with 70% Ethanol. Spin for 10 minutes. Pour off supernatant (**Be Careful!** When using silanized tubes pellet may not stick to bottom of tube).

19) Allow to air dry.

20) Resuspend each pellet in 200 m l TE (or appropriate volume for pellet). Allow to dissolve. Store at -20° C.

- Steps 1-8 taken from The Handy-Dandy DNA Methods Book Vol. 83, 122 of the H.D. Omniscience Training Course 1993 Handy-Dandy International: Steps 1-8 Tissue Extraction Protocol under Preparation of Highly Purified Plant DNA
- Steps 10-20 taken from a protocol obtained from QIAGEN by Hung Lee (ARS, USDA, WRRC Albany, CA)