gDNA Preparation from Plant Material using sorvall centrifuge

IMPORTANT NOTE: MAX SPEED OF MTM 6.4 ROTOR IS 4000 RPM!!!!

1. Picked one leaf from each plant (approximately 1 inch long or two smaller leaves). Put leaf into an appropriately labeled 1.2 mL microdilution stip-tube (USA Scientific; Cat # 1212-8000)
2. Grind plant tissue well with a stick into a powder, keep frozen placing tubes on liquid nitrogen.
3. Remove tubes from liquid nitrogen to warm up before adding extraction buffer.
4. Add 400 uL extraction buffer.
5. Stir the sample with the stick.
6. Vortex each tube really well for 30 seconds.
7. Spin tubes at max speed (4000 rpm, 21ºC) for 25 minutes.
8. Transfer supernatant (~200 uL) to a new tube using a pipet. **Pipet slowly** and avoid the leaf debris.
9. Add an equal amount (180 uL) of isopropanol and vortex well (10 seconds).
10. Incubate at room temperature for 15 minutes.
11. Spin tubes at max speed for 30 minutes.
12. Carefully remove supernatant with a pipette (do not pour off).
13. Wash pellet with 1 mL 80% ethanol. Vortex each sample for ~15 seconds. Note: possible stopping point. Pellet with ethanol may be stored in -20°C
14. Spin tubes for 10 minutes at max speed and pour off EtOH.
15. Spin again 2 minutes and carefully remove all traces of ethanol with a pipette.
16. Stick the tubes in the hood, either uncovered or with a kimwipe “tent” They dry much quicker (~30min)
17. Add 100uL of H2O and resuspend pellet by pipet and vortexing (disrupt the pellet as much as possible).
18. Spin at max speed for 10 minutes; transfer supernatant (this is the gDNA) to new 1.7mL tube
19. Store gDNA at -20 C.

Extraction Buffer Recipe:

REAGENT		[STOCK]	per/500 mL	per/100 mL
200 mM Tris, pH 7.5		1.0 M	100 mL	20 mL
250 mM NaCl		5.0 M	25 mL	5 mL
25 mM EDTA		0.5 M	25 mL	5 mL
0.5 % SDS		10%	25 mL	5 mL
Water	----		325 mL	65 mL