Extraction Protocol For Tropical and Temperate Plant Leaf Tissue

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Materials:
Qiagen Plant Mini Kit
Vortex mixer
1.5 mL Conical Microfuge Tubes
2mL Globe Scientific Round-Bottomed Tubes
P1000, P200, P10 Pipettors and Tips
Reagent Ethanol
Cleaning Ethanol
Cleaning Bleach
Scale
Weigh Boat
Tweezers
Scissors
Kimwipes
Tube Rack
5mm Titanium Beads
TissueLyser
Oscillating Dry Bath
Microfuge
Freezer Block Rack
Foam Microfuge Filler Blocks
Spoontula

Reagent Key:
Reagent 1 = AP1
Reagent 2 = RNAse
Reagent 3 = P3
Reagent 4 = AW1
Reagent 5 = AW2
Reagent 6 = AE

Steps:
1. Clean counter with bleach and ethanol
2. Add reagent ethanol to Qiagen reagents 4 and 5 according to their caps if not done already

3. Place tubes in rack thusly:
    a. 2 mL round-bottomed tube
    b. Lavender spin column
    c. 1.5 mL microfuge tube
    d. White spin column
    e. Empty waste tube
    f. 1.5 mL microfuge tube
4. Label tubes with IDs
5. Preheat oscillating dry bath to 65 degrees C and max oscillations. Take off plastic lid by sliding it backwards
6. Place 3 beads in round-bottomed tube
7. Shake loose silica from sample packet
8. Using tweezers take out leaf strips and weigh out 0.5 g using weigh boat and scale. Never use up all the sample, always leave at least a little material.
9. Using tweezers, hold each leaf strip over open round-bottomed tube and cut into thin bits using scissors. Do not add sand
10. Close tube and place in TissueLyser. Close lid very tightly and turn the unit on. Beat for 3 min at setting 50
11. Check that all samples have become powder, if any have not beat them again at 1 min intervals until they become powder
12. Check that there are no cracks in the round-bottomed tubes, if there are any transfer the powder and beads to a fresh tube. If you want to be safe you can do this for all tubes
13. Add 800 uL reagent 1 and 5 uL RNAse to round-bottomed tubes and place in oscillating dry bath for 30 min.
14. Take tubes out of oscillating dry bath and place in room temperature rack
15. Take freezer block rack out of -20 freezer and place behind your tube rack
16. Add 130 uL of reagent 3 to each tube, vortex, and immediately place tube in freezer rack.
17. Incubate tubes in freezer rack for 5 minutes
18. Centrifuge tubes for 8 min at 12,000 rpm
19. Transfer 600 uL supernatant to lilac spin column
20. Centrifuge lilac spin columns for 8 min at 12,000 rpm
21. Open all 1.5 mL microfuge tubes in row B and all white spin columns in row C
22. Transfer 500 uL of flow through to 1.5 mL microfuge tube avoiding disturbing the pellet
23. Add 750 uL reagent 4 to liquid in tube and mix immediately using pipette tip
24. Using same tip transfer ~600 uL mixture to lilac spin column and close it
25. Repeat 19, 20, and 21 for each sample
26. Centrifuge white spin columns for 4 minutes at 12,000 rpm
27. Discard flow through and repeat until all of the mixture has gone through the white spin column
28. Transfer spin basket to empty waste tube
29. Add 500 uL reagent 5 to each spin column
30. Centrifuge white spin column for 4 min at 12,000 rpm

31. Discard flow through and repeat steps 28 and 29
32. Transfer spin basket to empty 1.5 mL tubes
33. Add 50 uL reagant 6 directly on membrane of spin basket
34. Incubate at room temperature for 5 minutes
35. Centrifuge tubes at 12,000 rpm for 5 minutes with foam blocks on top of tube lids to prevent breakage
36. Repeat steps 32, 33, and 34
37. Close tube and use NanoDrop to quantify DNA and assess purity
38. Place tubes in labeled rack in -20 freezer
39. Clean beads
40. Clean counter with bleach and ethanol