CTAB-based DNA extraction from liquid N_2 macerated tissues.

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Materials needed:

Autoclave sterilized mortar and pestle.
Metal spatula (spoon)
2 ml microcentrifuge tubes
Pipettes (1mL, 200uL, 10uL)
Filter tips (1mL, 200uL, 10uL)

Chemical and enzymes needed:

CTAB Lysis Buffer pre-warmed at 60 °C
Phenol:Chloroform:Isoamyl alcohol (PCI)
Chloroform
RNase A and RNase If
Isopropanol
Ethanol
DEPC Treated DNase/RNase free water
Qubit HS DNA quantification kit
Agarose + PeqGreen

CTAB Buffer preparation:

Solution	**Stock Concentration**	**Final Concentration**	**Volume for 5mL**
CTAB	10%	2%	1 mL
PVP	10%	2%	1 mL
Tris-HCl pH 8.0	1 M	100 mM	500 uL
EDTA pH 7.5	500 mM	25 mM	2500 uL
NaCl	5 M	2M	2 mL
H_2O	-----	-----	150 uL
B-mercaptoethanol	-----	2%	100 uL

Procedure:

1. Transfer 3-4 spoons of N_2 macerate to a clean 2mL microcentrifuge tube.
2. Add 600 uL prewarmed **CTAB Buffer** and mix thoroughly by agitation and with the vortex.
3. On a thermomixer, incubate 30 minutes at 56-60 °C and 550 rpm. Mix thoroughly by agitation every five minutes.
4. Add 600uL (1 Volume) **PCI** and mix thoroughly by agitation for about 60 second. A milky liquid should form.
5. Centrifuge at >13000 rpm for 15 minutes.

6. Transfer as much of the upper phase as possible to a new, clean 2mL microcentrifuge tube.
7. Add 3uL **RNase A** and 2uL **RNase If** to the solution and mix carefully. Incubate on a thermomixer for 30 minutes at 37 °C.
8. Add 1 Volume (~ 500uL depending on much you recovered during the last step extraction step) of **Chloroform** and mix thoroughly by agitation; no milky solution will be formed but you should be able to see the formation of small droplets of chloroform.
9. Centrifuge at >13000 rpm for 15 minutes.
10. Using the 200uL (Yellow) Pipette, transfer max. 400uL of the upper phase to a new microcentrifuge tube.
11. Add 1 Volume (~400uL) **isopropanol** and mix carefully; you should see the nucleic acids forming a cloud and if yield is good some discrete particles forming.
12. Incubate at room temperature for 10 minutes.
13. Centrifuge at max. speed (>13000 rpm) for 20 minutes at 16-12 °C.
14. Remove the liquid with a pipette taking care not to disturb the pellet.
15. Add 1.5 to 2 volumes of fresh **ethanol** 70%-80%. Try to wash the walls of the tubes while adding the ethanol.
16. Centrifuge at max. speed (>13000 rpm) for 10 minutes at 16-12 °C.
17. Remove the liquid with a pipette taking care not to disturb the pellet.
18. Add 1.5 to 2 volumes of fresh **ethanol** 70%-80%. Try to wash the walls of the tubes while adding the ethanol.
19. Centrifuge at max. speed (>13000 rpm) for 10 minutes at 16-12 °C.
20. Remove the liquid with a pipette taking care not to disturb the pellet. In this step use the 10uL pipette to remove all remaining ethanol.
21. Let the pellet dry at room temperature for max. 10 minutes.
22. Add 30uL DEPC Treated, DNase/RNase free water.

Extraction quality control:

1. Run 3uL on a 0.5% agarose gel at 40V for ~4 hours. Use the Log-Ladder (Range: 10000bp→ 100bp) and the Lambda-HindIII marker (Range: ~23000bp→ 500bp) to check the quality of the DNA.
2. Use 2uL of the extract to measure the concentration of the DNA on the Nanodrop.
3. Use 1uL of the extract to measure the concentration of the DNA using the Qubit HS DNA kit.

Labeling of the extraction tubes:

The following information must be included on the extraction tube label:

On the tube lid:

DNA

On the tube side:

Organism
Details about experiment (if any)
Concentration in ng/uL
Date / Your Last Name

Example label:

Tethya wilhelma
LOW O_2, Replicate 1
152 ng/uL
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