Supplementary Material: Methods

CTAB DNA extraction protocol modified from Worden (2009)

Consumables:

1. CTAB Working Stock (48.5 mL pH 8.0)
    a. 1g CTAB
    b. 5ml 1M Tris-HCl
    c. 2ml 0.5M EDTA
    d. 14 ml 5M NaCl
2. RNAase A (pH 7.5)
    a. 10mg/ml in 0.01M Sodium Acetate
3. Proteinase K (filter sterilized)
    a. 10mg/ml in:
        i. 50% glycerol
        ii. 10mM Tris-HCl (pH 7.5)
        iii. 20mM $CaCl_2$
4. 10 mM Ammonium acetate in 76% ethanol
5. 80% ethanol (ice cold)

Master Mix CTAB (800 ul/rxn)

1. 776 ul CTAB Working Stock
2. 16 ul B-mercaptoethanol
3. 8 ul 10mg/ml Proteinase K

Protocol:

- Homogenize sample in 800 ul of Master Mix → Vortex, spin down
- Incubate @ 60°C for 1 Hr mixing often (every ~5 min)
- Add 800 ul Chloroform/Iso-amyl alcohol (24:1); Mix by inverting for ~2 minutes (Do altogether in rack)
    - *transfer samples to 2mL tube before adding Chl:Iso*
- Spin @ 14,000 x g for 10 minutes at 4°C → "Fast Cool" Button before step
- Carefully transfer ~600 ul of aqueous phase to clean 1.5ml tube → Used P-200
- Add 6 uL RNAase (from the 10mg/ml stock)
- Incubate @ 37°C for 30 min mixing often
    - Switch in new heat blocks to help lower temp
- Add 600 ul Isopropanol; Mix by gently inverting; Incubate for 2-24 hrs @ RT
- Spin @ 14,000 x g for 15 min at 4°C
- Carefully discard supernatant
- Wash pellet w/ 1ml 80% EtOH
- Incubate for 10 min. at room temp
- Spin @ 14,000 x g for 5 min at 4°C
- Carefully discard supernatant
- Wash pellet w/ 1ml of ice cold 80% ethanol
- Spin @ 14,000 x g for 5 min at 4°C
- Carefully discard supernatant
- Air dry on bench for 2-10 min
- Resuspend in 30 ul H_2O; Store sample at -20°C

Check Nanodrop: 260/280 = 1.8-2.0; 260/230 > 1.8