CTAB DNA Prep of *A. dissimilis*
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CETYLTRIMETHYLAMMONIUM BROMIDE BUFFER. Extraction of nucleic acids was performed following a method described by Vierstraete (2009) and Sambrook et al. (1989), somewhat modified:

1. Add 500 µl CTAB buffer (see recipe below) to an animal in a microcentrifuge tube.
2. Crush the worm with a sterile micropestle while keeping the tube in an ice bath.
3. Heat the tube to 95℃for 10 min, freeze in liquid nitrogen, vortex for 15 seconds.
4. Repeat step 3 2X.
5. Centrifuge at 13,000 rpm for 7 minutes at 4℃.
6. Transfer the liquid phase to a new microcentrifuge.
7. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, pH=8) and mix by flicking the tube. Centrifuge for 5 (?) minutes at 13,000 rpm.
8. Transfer upper phase to a new tube. Add an equal volume of chloroform/isoamyl alcohol (24:1 v/v), vortex, and centrifuge for 5 (?) minutes at 13,000 rpm. Transfer the upper phase to a new tube.
9. Add 750 µl cold isopropanol and 1/10th volume sodium acetate. (**what concentration?)**
10. Store tubes at -20℃overnight.
11. Centrifuge for 30 minutes at 13,000 rpm, 4℃.
12. Decant the supernatant.
13. Was the DNA pellet in 200 µl cold 70% ethanol.
14. Air dry the DNA pellet.
15. Resuspend in 50 µl of PCR-grade water.

Clay purchased CTAB solution (cat# 786-564) from G-Biosciences and they provided the following protocol:

STORAGE CONDITIONS Shipped at ambient temperature. Upon arrival, store at room temperature. ADDITIONAL ITEMS REQUIRED • PVP (Polyvinylpyrrolidone) (Cat. # RC-085, RC-086)

PREPARATION BEFORE USE For plant samples, dissolve 1% (w/v) of PVP into the required volume of CTAB Extraction Solution (0.6g in 60ml).

PROTOCOL

1. Pulverize 100 mg of sample using a liquid nitrogen chilled mortar and pestle. Once processed, mix 100 mg of frozen powdered sample with 500 µl of CTAB Extraction Solution. NOTE: Samples can be ground with pestle and mortar with suitable grinding resin (Molecular Grinding Resin, 786-138PR), in lieu of liquid nitrogen.

    Place tube w worm in LN2 bath to freeze. Or maybe drip in some LN2? Then grind w mortar and pestle from Morimoto lab.

2. Place the homogenate into a 60°C bath for 30 min.
3. Centrifuge the homogenate for 10 minutes at 10,000 x g.
4. Transfer the supernatant into a clean tube and add 5 µl of RNase (10 mg/ml in water) to the lysate. Incubate at room temperature for 15 minutes.
5. Centrifuge for 5 minutes at 10,000 x g.

6. Extract the lysate with equal volume of chloroform: isoamyl alcohol (24:1). Vortex for 5 seconds then centrifuge for a minute at 10,000 x g to separate the phases.
7. Transfer the upper phase to a clean tube.
8. Repeat step 7 until upper layer is clear. Transfer upper phase to a new tube.
9. Add 0.7 volumes of isopropanol. Mix and incubate at -20°C for 15 minutes.
10. Centrifuge for 10 minutes at 10,000 x g. Decant and wash the pellet with 70% ethanol.
11. Decant without disturbing the pellet. Dry the pellet briefly in a speed vac or at room temperatuer. Do not over-dry the DNA.
12. Resuspend the DNA in 50 µl of TE buffer