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Protocol status: Working
We use this protocol and it's working

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Mycota Lab CTAB Protocol

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ABSTRACT

This protocol is an amalgamation of the methods used by Osmundson et al. and Forin et al. for fungal herbarium specimens.

MATERIALS

CTAB extraction buffer – Cepham Scientific - $92.25/ 500mL - $0.1845 / sample
Stainless Steel Beads 2mm – Amazon - $5.49 / 800 beads
1.5mL Eppendorf Lo-Bind Tubes – USA Scientific - $39.70 / 250 tubes - $0.63 / sample
Proteinase K – MPBio - $101.65 / 10mL – Makes 4000 samples - $0.025 / sample
RNASE A – Sigma (E6148-25ML) -$210.00 / 100mg - Makes 4000 samples - $0.052 / sample
phenol:chloroform:isoamyl alcohol (25:24:1) – Sigma (P3803-400ML) - $295.00 / 400mL – 500 samples - $0.59 / sample
chloroform:isoamyl alcohol (24:1) – Sigma (25668-500mL) - $430.00 / 500mL – 830 samples - $0.52 / sample
70% Ethanol – IBI Scientific
Mag-Bind TotalPure NGS Beads – Omega Biotek - $122.60 / 5mL – 2500 samples - $0.049 / sample

Total Cost / Sample = ~$2.25 / sample

For reference:

		Quantity (24 mL lysis buffer)	Quantity (48mL lysis buffer)
100 µg/mL	RNAse A (20 mg/mL)	120 µL	240 µL
100 µg/mL	Proteinase K (20 mg/mL)	120 µL	240 µL

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DNA Extraction Procedure

1 Create your stock Proteinase K and RNASE A solutions. Both at 20mg / mL. So for 100mg of Proteinase K, add 5 mL of molecular water. Same for the RNASE A.

2 Prep your CTAB buffer in 50mL tubes. Add 500 uL of CTAB buffer per sample. 96 samples add 48mL of CTAB. For this amount of CTAB add 240 uL of Proteinase K and 240uL of RNASE A.

3 Place fungal tissue and a stainless steel bead into a 1.5mL Eppendorf tube.

4 Add 1mL of prewarmed (65◦C) CTAB extraction buffer with the addition of proteinase K (1 mg/mL) and RNase A (10 mg/mL).

5 Apply three rounds of freeze-thaw consisting of alternating three min treatments in dry ice and a 70uC heating block, followed by a (30 minutes to overnight) incubation at 70◦C.

6 Centrifuge for 10 minutes at max speed.

7 Transfer the liquid phase to a new 1.5 mL microcentrifuge tube.

8 Add 800 µL of phenol:chloroform:isoamyl alcohol (25:24:1). Invert the tube 15 times to mix.

9 Centrifuge for 10 minutes at 10,000 rpm.

10 Move the upper liquid phase to a new 1.5mL tube and add 600 µL of chloroform:isoamyl alcohol (24:1). Invert the tube 15 times to mix.

11 Centrifuge for 10 minutes at 10,000 rpm.

12 Precipitate the DNA by adding ice-cold isopropanol (2/3 of the recovered volume) and incubate at −20◦C for 2 hours.

13 Centrifuge for 10 minutes at 10,000 rpm. Pour off isopropanol.

14 Add 300 µL of ice-cold 70% ethanol to wash. Let sit for 2 minutes.

15 Centrifuge for 10 minutes at 10,000 rpm. Pour off the ethanol.

16 Repeat the previous ethanol wash a second time.

17 Let the pellet air dry for at least 15 minutes or until all the ethanol has evaporated.

18 Resuspend the pellet in 20uL of molecular water. Incubate overnight?

19 Bead cleanup. Add 20uL of beads to the tube. Wait 5 minutes. Put on magnet for 2 minutes. Remove liquid. Add 125mL of ethanol. Wait 2 minutes. Remove ethanol. Repeat wash. Let dry 1 minute.

20 Add 20uL of molecular water.

21 Store the DNA at -20C.