**CTAB Fungal DNA Extraction Protocol**

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\*Adapted from Cubero et al., 1999 and Lagonigro et al., 2004

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**Reagents Needed:**

Liquid Nitrogen

CTAB Extraction Buffer + PVP

CTAB Precipitation Buffer (pH=7.2-7.6)

24:1 Chloroform: Isoamyl alcohol

100% Isopropanol (ice cold)

70% Ethanol (ice cold)

100% Ethanol (ice cold)

3M Sodium Acetate (pH=5.2)

5M NaCl

UltraPure water or TE buffer

Proteinase K 20 mg/mL

RNase A 10 mg/mL

**Instruments/Materials Needed:**

Bead beating tubes

Glass beads

Dewar for Liquid Nitrogen

Water bath or heat block set to 70° C

1.5mL Microcentrifuge tubes

2.0mL Microcentrifuge tubes

15mL Centrifuge tubes

Fridge and Freezer (4° C and -20° C)

Pipettes

Refrigerated Microcentrifuge

Fungal samples, **0.03g** minimum

**Recipes:**

CTAB Extraction Buffer + PVP

1% w/v CTAB

1M NaCl

100mM Tris

20mM EDTA

\*RIGHT BEFORE USE ADD: 1% w/v Polyvinyl pyrrolidone (PVP)

\*RIGHT BEFORE USE ADD: 1% of 20mg/mL Proteinase K

CTAB Precipitation buffer:

1% w/v CTAB

50mM Tris-HCl

10mM EDTA

40mM NaCl

\*Adjust pH to 7.2-7.6

**Protocols:**

Fungi growing in liquid sample prep:

1. Grow fungi in preferred media type till fungi are in mid-exponential phase
2. Either filter fungi through a vacuum filter funnel and filter paper, or centrifuge fungal samples and wash with water 3 times, then obtain as tight of a fungal pellet as possible and remove all liquid from the tube

Fungi grown on solid sample prep:

1. Grow fungi on preferred media type till fungi grows to its maximum on the plate
2. Place 800µL of water in a 1.5mL tube and weigh recording the weight
3. Scrape 0.03g of fungus off the plate and place into the tube, avoiding agar
4. Centrifuge the tube to obtain a fungal pellet and remove all water

Lysing fungal cells for FILAMENTOUS FUNGI

1. Grab as many mortar and pestles as necessary for the number of samples you have (or wash them out thoroughly with 70% ethanol between samples)
2. Obtain liquid nitrogen placed in Dewar
3. Place tubes with processed fungi in a small container and pour liquid nitrogen over the tubes until the fungi pellets are frozen completely
4. With tube lids closed, tap the lids against the table to dislodge the pellet from the tube
5. Put the frozen fungal pellet into the mortar and pour additional sterile Liquid nitrogen over the pellet
6. Carefully grind the fungus being sure not to launch it out of the mortar, grind till the fungus is a powder, adding more liquid nitrogen if the fungus gets too liquidy
7. Scrape ground up fungus out of the mortar and place in a fresh 1.5mL tube, place sample in -80° C freezer till ready for DNA extraction.
8. Repeat process with all samples.

Lysing YEAST fungal cells:

1. Grab as many bead beading tubes as you have samples
2. Pour ~300uL of glass beads into tubes; and label tubes accordingly
3. **PIPETTE PREPARED DNA EXTRACTION BUFFER INTO BEAD BEATING TUBES**
4. Take 1.5 mL tube of washed cell pellet and place in liquid nitrogen to flash freeze
5. Dislodge pellet from bottom of tube by tapping tube on a table
6. Transfer fungal pellet into bead beating tube

DNA extraction Protocol:

1. Day -3 to -7:
2. Grow fungi in preferred media type till fungi are in mid-exponential phase (3-7 days)
3. Day 1: Prepare samples
4. Pipette 1 mL of fungus into a 1.5 mL tube
5. Centrifuge fungal samples at 10,000x g for 1 minute
6. Wash with 1 mL of ddH2O 3 times centrifuging in between, then obtain as tight of a fungal pellet as possible and remove all liquid from the tube
7. Take your 1.5 mL tube of washed cell pellet and place in liquid nitrogen to flash freeze
8. Place tubes in -80 freezer till you’re ready to extract DNA
9. Day 2: DNA extraction
10. Turn on water bath/heat block to 70° C
11. Determine the number of samples you will be processing, multiply that number by 750 µL to determine the amount of CTAB Extraction buffer you will need (700 µL per sample) and aliquot that out into a 15 mL centrifuge tube
12. **Add 1% w/v PVP (.01g/mL) and 1% v/v 20mg/mL Proteinase K (5µL/500µL) to your aliquoted CTAB Extraction buffer**
13. Grab as many bead beating tubes as you have samples
14. Pour ~300uL of glass beads into tubes and label tubes accordingly
15. Remove tubes of frozen fungus from the freezer
16. **Dislodge pellet from bottom of tube by pipetting 700 µL of the complete CTAB Extraction buffer into each frozen sample tube**
17. Transfer sample in CTAB Extraction buffer to Bead beating tube
18. Put tubes in 70 °C water bath or heat block for 5 minutes, then bead beat for 5 minutes; repeat 2 more times (30 mins total) (change temperature to 37° C after this step, unless you have a 37° C incubator)
19. Confirm cells have lysed via microscopy, 5 µL of sample onto a microscope slide
20. Remove tubes from the water bath/heat block and transfer as much liquid as possible into a new 2 mL microcentrifuge tube, avoiding the glass beads
21. Add 1x the volume of 24:1 Chloroform: Isoamyl to the tubes, invert tubes to mix and then centrifuge tubes at 10,000x g for 5 minutes
22. Carefully remove tubes from centrifuge, collect upper layer only, and transfer the upper layer into a new 2 mL microcentrifuge tube
23. Add 2 volumes of CTAB Precipitation buffer to the tubes and mix by inverting for 2 minutes
24. Centrifuge tubes at 13,000x g for 15 minutes
25. Remove supernatant, being sure to leave the pellet behind in the tube
26. Re-suspend pellet in 350 µL of 1.2M NaCl
27. Add 2 µL of 10 mg/mL RNase A to tubes and place tubes at 37° C for 30 minutes
28. Remove tubes from 37° C and add one volume of 24:1 Chloroform: Isoamyl; mix thoroughly by inverting
29. Centrifuge tubes at 10,000x g for 5 minutes
30. Remove upper phase and transfer to a new 1.5 mL tube
31. Add 0.6 volumes of ice cold isopropanol and mix by inverting
32. (PAUSE POINT) Place tubes in -20 °C freezer for 15 minutes OR OVERNIGHT/FOR MULTIPLE DAYS
33. Centrifuge tubes in 4 °C at 13,000x g for 20 mins
34. Remove all liquid and let air dry for 30 mins - **till it’s dry** to remove excess alcohol
35. Resuspend pellet in 30-50 µL of UltraPure water or TE buffer

DNA clean-up Protocol (only necessary if 260/280nm is below 1.8):

1. Add 0.1 volumes of 3M Sodium Acetate and 3 volumes of ice cold 100% Ethanol to your DNA samples, mix by inverting.
2. Freeze samples at -80° C for 20mins or at -20° C overnight.
3. Centrifuge tubes at 4° C at max speed for 30 minutes
4. Remove supernatant and resuspend pellet in 1mL of ice cold 70% Ethanol, being sure the DNA pellet becomes loose from the tube and floats freely in the liquid.
5. Centrifuge at 4° C at max speed for 10 minutes
6. Remove Ethanol and let tubes dry completely with no ethanol left in the tubes. Overnight with running air over the open tubes works best.
7. Pre-heat elution buffer (TE or water) at 65° C, then re-suspend DNA in 30-50µL of warmed elution buffer

Removal of Melanin from DNA from Lagonigro et al., 2004:

**Solutions:**

5M NaCl

7M guanidine hydrochloride

CTAB-Urea buffer

* + 50 mM Tris-HCl, pH 7.0
  + 1% CTAB
  + 4M Urea
  + 1 mM EDTA

**Procedure:**

1. Water is added to ~ 100- 200 µL DNA/RNA solution until a volume of 400 µL is reached.
2. 130µL 5M NaCl is added and 1.6 mL of CTAB-Urea solution
3. Samples are mixed (by hand) and incubated o/n at 4°C
4. Samples are centrifuged for 15 minutes at max speed at 4°C
5. Precipitated RNA/DNA is resuspended in 400 µL 7M guanidine hydrochloride
6. Add 2 Vol of EtOH (100%)
7. Incubate on ice for 1 hour
8. Centrifuge for 15 min at 4 °C
9. Wash twice with 70% EtOH
10. Centrifuge 10 min at max speed at RT
11. Remove supernatant, dry pellet an resuspend in TE

References:

1. Cubero, O. F., Crespo, A. N. A., Fatehi, J., & Bridge, P. D. (1999). DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. *Plant Systematics and Evolution*, *216*(3-4), 243-249.
2. Lagonigro MS, De Cecco L, Carninci P, Di Stasi D, Ranzani T, Rodolfo M, Gariboldi M: CTAB-Urea method purifies RNA from melanin for cDNA microarray analysis. *Pigment Cell Res*2004, 17:312-315.