Modified CTAB Extraction of Fungal DNA (for Herbarium Samples)

*Turn on 65˚C water bath.

1. Cut a small piece of tissue from lesions on herbarium specimens and place in 1.5 ml tube.
2. Add 150 µl **Extraction Buffer** and grind with sterile Konte pestle.
3. Add 150 µl **Nuclei Lysis Buffer** and 60 µl **5% Sarkosyl**, vortex to mix.
4. Incubate at 65˚C for 30 min.
5. Add 1V (~300 µl) **Chloroform:Isoamyl alcohol (24:1)**, invert several times to mix.
6. Centrifuge at 12,000 rpm for 15 min at RT.
7. Transfer aqueous phase to a new tube.
8. Repeat steps 5, 6 & 7.
9. Add 5 V (1250 µl) of **Buffer PB** to aqueous phase and mix.
10. Bind DNA to column by applying vacuum (800 µl maximum volume at a time).
11. Wash column with 750 µl of **Buffer PE** and apply vacuum.
12. Transfer column to a 2 ml collection tube and centrifuge for 1 min at 13,000 rpm to remove residual ethanol in Buffer PE.
13. Place each column into a clean 1.5 ml tube.
14. To elute DNA, add 30 µl of **Buffer EB** (10 mM Tris-HCl, pH 8.5) to the center of each membrane, let it stand for 1 min, and then centrifuge for 1 min at 13,000 rpm.
15. Store DNA at -20˚C.

Reagents:

Extraction Buffer: 1L

0.35 M Sorbitol	63.77 g
0.1 M Tris	12.11 g
0.005 M EDTA	1.86 g

Adjust pH to 7.5 with HCl.
Add 0.02 M Sodium Bisulfite (3.8 g/L) just before use.
Do not autoclave and store at 4˚C.

CTAB (Nuclei Lysis Buffer): 1L

0.2 M Tris, pH 7.5	24.22 g
0.05 M EDTA	18.61 g
2.0 M NaCl	116.88 g
2% CTAB (cetyltrimethylammonium bromide)	20.00 g

Adjust pH to 7.5 with HCl.
Autoclave.

5% Sarkosyl:

5 g of N-lauryl sarcosine for 100 ml final volumn
Autoclave.

Chloroform:Isoamyl alcohol (24:1)

QIAquick PCR Purification Kit contains:
Buffer PB
Buffer PE – Add ethanol (96-100%) to Buffer PE before use
Buffer EB (10 mM Tris-HCl, pH 8.5)