Cryptococcus neoformans DNA Extraction Method

Based on Pitkin *et al*. (1996) *Microbiology* 142: 1557-1565. Results in ~0.5-2 mg DNA.

1. Grow a 50 ml YPD overnight culture shaking at 30°C.

2. Pellet cells in tabletop centrifuge in a 50 ml disposable tube. [Optional: wash pellet with water and repeat spin].

3. Freeze cells at –20-80°C for <30 min, then dry in a freeze drying machine.

4. Add the equivalent of 3-5 mL of 2 mm glass beads and vortex/shake until the cell pellet is broken and a fine powder created.

5. Add 10 ml CTAB extraction buffer (see below) and mix. Do this in the fume hood.

6. Incubate at 65°C for 30 min.

7. Add 10 ml chloroform (in fume hood) and gentle mix for a minute or so.

8. Spin in a table top centrifuge for 10 min (2,500 – 3,000 rpm).

9. Remove supernatant (c. 7 ml) and add to an equal volume of isopropanol in a 15 ml disposable tube.

10. Gently rock back and forward to mix. If the DNA precipitates in strands and clumps, spool out with a glass pipette and transfer to eppendorf containing 1 ml 70% ethanol. Otherwise, spin in a table top centrifuge for 10 min, pour off supernatant and use 1 ml 70% ethanol to wash DNA pellet and transfer it to an eppendorf.

11. Spin sample in microcentrifuge for 5-10 min. Remove ethanol and allow to air dry.

12. Resuspend DNA in either water or TE buffer (c. 500 μl). RNase can be added to final conc. of 20 μg/ml if needed. Run 1 μl on an agarose gel to check conc. and quality.

Extraction buffer (100 ml)

Stocks solution	Add	Final concentration
1 M Tris-HCl, pH 7.5	10 ml	100 mM
5 M NaCl	14 ml	0.7 M
0.5 M EDTA	2 ml	10 mM
CTAB powder	1 g	1%
β-mercaptoethanol (14 M)	1 ml	1%
Water	73 ml	

CTAB is mixed alkyltrimethyl ammonium bromide, Sigma cat.# M7635. This takes time to go into solution. You can also use solid NaCl rather than a 5 M solution if that is easier. The buffer lasts four-six months at room temperature. If one adds the β-mercaptoethanol just prior to using it then it seems to work better for long strands and spooling.