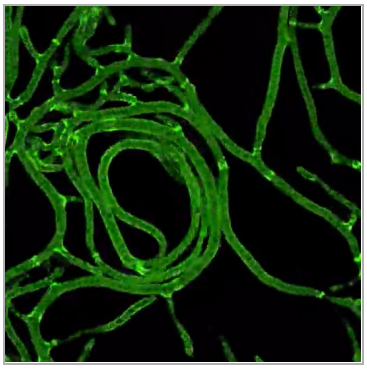

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MANUSCRIPT CITATION:
Based on Pitkin et al. (1996) Microbiology 142: 1557-1565. Results in ~0.5-2 mg DNA.

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

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Cryptococcus neoformans DNA Extraction Method

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ABSTRACT

Extraction method to obtain genomic DNA from Cryptococcus neoformans.

ATTACHMENTS

DNAextraction_Southerns.pdf

GUIDELINES

Extraction buffer (100 ml)

	Stock 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	2ml	10 mM
	CTAB powder	1g	1%
	B-mercaptoethanol (14 M)	1ml	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.The buffer seems to work better for long strands and spooling if β-mercaptoethanol is added just prior to use.

SAFETY WARNINGS

See SDS (Safety Data Sheet) for hazards and safety guidelines.

1 Grow a 50 mL YPD culture overnight (16:00:00), shaking at 30 °C .

2 Pellet cells in tabletop centrifuge in a 50 ml disposable tube.

Note

Optional: wash pellet with water and repeat spin.

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

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

5 **In fume hood**, add 10 mL CTAB extraction buffer (see Guidelines) and mix.

6 Incubate at 65 °C for 00:30:00 .

7 **In fume hood**, add 10 mL chloroform and gently mix for approximately 00:01:00 .

8 Spin in a table top centrifuge for 00:10:00 (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 00:10:00, pour off supernatant and use 1 mL 70% ethanol to wash DNA pellet and transfer it to an eppendorf tube.

11 Spin sample in microcentrifuge for 5-10 minutes. 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 concentration of 20 µg/ml if needed.

Run 1 µL on an agarose gel to check concentration and quality.