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Small scale DNA preps for *Neurospora crassa*

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Small scale DNA preps for *Neurospora crassa*

Abstract

Molecular biology experiments often require preparation of small amounts of DNA from many samples. This abbreviated DNA isolation method yields an average of 0.6 micrograms of genomic DNA that is suitable for Southern analysis or PCR. Starting with fresh mycelium, 20 to 40 samples can be processed in approximately two hours. Better yields (about 5 micrograms) may be obtained by suspending approximately 100 microliters of ground lyophilized mycelium in 500 microliters of isolation buffer and following the protocol starting from step 4. "Spin" refers to centrifugation of samples at 14,000 rpm in a microcentrifuge.

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1. Inoculate 500 microliters of liquid growth medium (Vogel's, 1.5% sucrose) in 1.5 ml microcentrifuge tubes that have had an air hole poked in the top of each with a 20 gauge needle. Shake for one to two days at 34 degrees C, or until the cultures are saturated.
2. Spin the culture tubes for five minutes to compress the mycelia, then invert and blot the tubes on a paper towel to remove most of the supernatant.
3. Add approximately 150 microliters of Ottawa Sand (Fisher Scientific # S-23) plus 500 microliters of isolation buffer (50 mM Tris-HCl pH 8, 170 mM EDTA pH 8, 1% N-lauroylsarcosine) to each tube and vortex for two minutes. More complete mixing may be achieved by taping the tubes to close the air holes, inverting, and vortexing for an additional one minute.
4. Incubate the tubes at 65 degrees C for 5-7 min.
5. Add 300 microliters of 7.5 M ammonium acetate, invert each tube to mix the contents and incubate on ice for 10 min.
6. Spin for 3-5 min and pipette 700 microliters of the supernatant into a new tube.
7. Add 500 microliters of isopropanol to each sample, invert the tubes to mix the contents and incubate on ice for 10 min.
8. Spin for 3 min, discard the supernatant and invert the tubes on a paper towel to wick away the excess liquid.
9. Add 250 microliters of TE (may include RNase here) to each pellet and incubate at 50 degrees C for 5-10 minutes (vortex briefly if the pellet doesn't dissolve completely).
10. Add 200 microliters of chloroform:isoamyl alcohol (24:1), vortex briefly and spin for one minute. Collect the aqueous phase (approximately 220 microliters). This is a good stage to check the concentration of DNA.
11. Ethanol precipitate the DNA and resuspend to a convenient concentration.