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A moderate-scale DNA prep for Neurospora.

Several methods for preparing DNA from Neurospora have been reported and are in current use (Metzenberg and Baisch 1981 Neurospora Newsl 28:20-21; Stevens and Metzenberg 1982 Neurospora Newsl. 29:27-28, Feher and Schablik 1983 Neurospora Newsl. 30:14; Vollmer and Davis 1985 Neurospora Newsl. 32:16-17). These procedures, while yielding usable DNA, in our hands all have certain drawbacks: either they are not easily scaled up or yield DNA that is refractory to digestion by certain restriction enzymes. We have combined steps from several of these methods and from a procedure for purification of Aspergillus DNA (Yelton et al. 1984 PNAS 81:1470-1474). The following protocol yields a substantial quantity (200-300 ug) of fairly pure, easily cuttable DNA when either logarithmically growing hyphae or a post-logarithmic pad is used. The DNA obtained is greater than 50 kb in length. Given enough mortars and pestles, a dozen samples can be conveniently processed in one day.

1. Inoculate a 40 ml culture (Vogel's medium) with 10^6 conidia/ml. Grow 40-48 h at 25°C with shaking.
2. Filter the mycelia on a Buchner funnel and wash with H2O.
3. Transfer the mycelial pad to a pre-chilled (-70°C) mortar, add liquid N2, and grind to a very fine powder.
4. Transfer the powder to a 50 ml polypropylene tube and add 15 ml 5 mM EDTA pH 8.5 - 0.2% SDS, and 15 ul diethylpyrocarbonate (DEPC). Shake vigorously only long enough to dislodge the frozen material, then continue to invert slowly for one minute.
5. Incubate at 70% for 15 min.
6. Chill on ice at least 10 min. (If multiple samples are to be processed on the same day, tubes can be left on ice for up to 1 h in order to synchronize all the samples.)
7. Add 0.95 ml 8M potassium acetate pH 4.3. Incubate on ice 1 h.
8. Centrifuge at 14,000 rpm for 15 min, at 4°C.
9. Transfer the supernatant to a new tube. Add 15 ml isopropanol and mix gently. A clot should immediately form.
10. Rinse pellet with 70% ethanol. Drain well and air dry for 10 min.
11. Resuspend pellet in 4 ml 1 mM EDTA pH 8.0. Add 2 ml High Salt Buffer (Metzenberg and Baisch, op. cit.) plus 15 ul (boiled) 10 mg/ml RNase A. Incubate at 37°C for 30 min.
12. Add 180 ul 100 mM spermine-4HCl. Mix gently, then incubate on ice 20 min. Pipet off the liquid from the clotted DNA.
13. Rinse the pellet 3x on ice, for 30 min. each, with 2 ml cold spermine wash buffer (75% ethanol, 10 mM Mg acetate, 0.3 M Na acetate, pH 6.0), pipetting off rinse each time.
14. Rinse briefly with 70% ethanol. Drain well.
15. Air dry 10 min, then add 1 ml 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.1 M NaCl. Allow to resuspend overnight at 4°C.
16. Reprecipitate the DNA with 2 ml ethanol at room temperature. A clot will immediately form. Pipet off the supernatant, rinse pellet with 70% ethanol, air dry 10 min., and resuspend in 1 ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)

Comments:

1. We have omitted the DEPC in step 4 on occasion with no ill effect.
2. Smaller quantities of log phase mycelia may also be processed by this protocol. It is not necessary to scale down solution volumes for pads half the size used here. In general, we no longer bother to accurately measure the conidial inoculum and simply harvest one 14 x 150 Vogel's slant tube to inoculate.

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