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Abstract

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We have developed a procedure for small-scale DNA isolation from Neurospora for use in screening multiple isolates. It is faster than the procedure reported by Metzenberg and Baisch (Neurospora Newsl.28:20-21,198l). The cell wall of Neurospora has a polycation, polygalactosamine, that binds long-chain polyphosphate (Harold, F. M. and A. Miller, Biochim, Biophys, Acta 50: 261-270, 1961), and we reasoned that it might bind DNA in phenol extraction procedures. We therefore tried to isolate DNA from exponentially growing cells, with many nuclei and thin cell walls; losses of DNA which might bind to wall polycations might be tolerable. The method described below has worked well for us, but it is by no means perfected. It is offered as an idea for further improvement, somewhat prematurely, owing to the annual publication schedule of the Newsletter.

The method yields DNA of good quality, indicated by restriction endonuclease digestions, and has an average size of 65-70 kb. The yield approaches 1 µg DNA per mg dry weight of culture. The entire procedure, from inoculation to analysis by gel electrophoresis, requires approximately 24 hr.

PROCEDURE: 1. Start 20- to 40-ml cultures, inoculated with 1 x 10^6 conidia/ml, and allow them to germinate at 25 °C overnight (12 hr, with strong shaking or aeration) to a dry weight of about 0.3 to 0.8 mg per ml. Collect cells and wash on a filter funnel (we use l-inch circles of Whatman filter paper). Disperse in 4-5 ml 1M sorbitol in a 30-ml Corex tube. Avoid aggregated mycelial growth that might occur on the rim of the medium; evenly dispersed mycelium works well.

2. Add 1 ml Novozym reagent and incubate for 60 min at 30° C. Gentle agitation may help.

3. Add 6 ml hot (70° C) Phenol reagent and vortex very briefly (2-3 set). Place on ice for 10 min. Centrifuge 5 min at 4500 x g.

4. Remove most of upper phase (ca. 7.5 ml) to a clean Corex tube. volumes (15 ml) ethanol. Centrifuge 5 min at 7500 x g.

5. Disperse pellet in 800 µl 1 mM Na_2EDTA (pH 8.0). Add 400 µl high-salt buffer containing 50 µg boiled RNase A. Incubate 30 min at 37° C. Place on ice.

6. Add 100 mM spermine (dissolved in TE) to bring extract to 3 mM spermine. Incubate on ice for 20 min. A clot should form quickly and condense (and sink) gradually. Carefully remove the hazy supernatant with a pipette, leaving clot. Add 1 ml cold spermine wash buffer to the clot and incubate on ice for 30 min; change solution in the same manner and incubate another 30 min. Remove supernatant with a pipette, leaving DNA clot. (A longer incubation in the second wash may be in order, particularly if the procedure is scaled up.)

7. Wash clot with cold 70% ethanol, centrifuge briefly, and carefully remove ethanol wash. Remove residual ethanol by vacuum centrifugation or heating at 65° C for 15 min. (Fully dry pellets will be hard to redissolve.) Dissolve pellet in 80 µl 1 mM Na_2EDTA, pH 8.0, then add 20 µl 5X high-salt buffer.

REAGENTS: 1. Novozym reagent: In 1M sorbitol disperse 2 mg/ml dry Novozym 234 powder (Novo Laboratories, Inc., 59 Danbury Rd., Wilton, CT 06897). Make fresh and keep on ice.

2. Phenol reagent: Add 150 ml of a solution which is 0.1 M Tris HCl, pH 8.0, 0.67 M NaCl and 1% sodium dodecyl sulfate to 100 g purified crystalline phenol. Heat to 65°C to allow the phases to mix fully. Divide into 50-ml aliquots and store at -20°C.

3. Spermine wash buffer: 75% ethanol, 10 mM Mg acetate, 0.3 M Na acetate, pH 6.0. Store at 4° C.

4. High-salt and 5X high-salt buffer are those of Metzenberg and Baisch (op.cit.).

COMMENTS: We have found this method to be fast and reliable. Despite repeated attempts, however, we have not been able to use this procedure to isolate DNA from late-log or stationary (mycelial) cultures. Critical steps in the procedure are the use of spermine to condense selectively large pieces of nucleic acid (Hoopes, B. C. and W. R. McClure, Nucl. Acids Res. 9: 5493-5504, 1981), and aspiration of the supernatant, rather than centrifugation, to collect precipitated material. A benefit of rapid cell growth is the many mitochondria in these cells: distinct patterns of mitochondrial DNA bands are visible, superimposed on the diffuse nuclear DNA digestion products, when restriction endonuclease reaction products are separated by agarose gel electrophoresis. These bands match exactly those seen after digestion of purified mitochondrial DNA (Taylor, J. W. and B. D. Smolich, Curr. Genetics 9: [in press], and they may be used as internal standards. We expect that this method can be scaled up to yield milligram quantities with suitable modifications. (Supported by NSF Grant PCM82-08866 to C. Yanofsky. S.J.V. is a Fellow of a Career Investigator of the American Heart Association). - - - Department of Biological Sciences, Stanford University, Stanford, CA 94305. [R.H.D. on leave from the Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, CA 92717.1