Metzenberg, R. L. and.T. J. Baisch

An easy method for preparing Neurospora DNA.

We have been studying the segregation of certain DNA "restriction site markers" in Neurospora crosses. The method of Hautala et al. (1977 J. Bacteriol. 130: 704), slightly modified, yielded DNA of good quality but was much too laborious for preparing large numbers of samples. The following method works well in our hands and allows routine preparation of 30-40 DNA samples at a time.

Reagents: "Extraction buffer" is 250 mM lithium salt of ethylenediamine tetraacetate, pH 8.0, 0.5% w/v Triton X-100, and 250 µg/ml of Pronase (Sigma bacterial protease). It is made up fresh by mixing the following stock solutions. Li_3EDTA (500 mM) is prepared by neutralizing the free acid of EDTA (Fisher Scientific Co.) with solid $LiOH.H_2O$ to pH 8.0 (this requires about 3.6 equivalents): the solution is stored at room temperature. The somewhat exotic salt, Li3EDTA, is used because, unlike Na_3EDTA, it does not come out as a syrup from ethanolic solutions. Triton X-100 is stored frozen as a 10% (w/v) stock solution. Pronase (Sigma protease, type VI), 2 mg/ml in 10 mM Tris buffer, pH 7.5, is predigested l-2 h at 25°C and stored frozen. "Ethanolic perchlorate reagent" modified slightly from the description of Wilcockson (1975 Anal. Biochem. 66: 64) is made up as follows. $NaC1O_4.H_20$ (140 g.) plus 36 ml of water are warmed carefully to 60-70°C (perchlorate is a powerful oxidizing agent; warm it only in glassware). The warm solution is filtered through a glassfiber filter to remove bits of dust, and then 381 ml of 100% EtOH are added immediately. The resulting solution is nearly saturated at room temperature. Ethanolic perchlorate is a chaotropic agent which denatures proteins and leaves most of them in solution, while precipitating nucleic acids; we have found it to be much easier to use than phenol, and have had no problems with residual enzyme inhibitors left in the final preparations. "Low-salt buffer" is 1 mM NaCl + 1 mM Tris.HCl, pH 7.4, + 0.1 mM Na2EDTA. "High-salt buffer" has the same ingredients at 100 mM, 25 mM, and 2 mM respectively.

Neurospora is grown in 20 ml of Vogel's or other medium, with or without shaking, to stationary phase. Mycelial mats are harvested by vacuum filtration, washed with a little-water, and the damp mats are stacked between numbered filter papers and dried in vacuo. The dry pads (25-75 mg each) are crumbled into round-bottom glass centrifuge tubes (5-10 ml) and three glass beads (4 mm diam.) are put into each tube. The mycelia are pulverized for about 20 sec by holding the tube against a Vortex mixer running at full speed.

Then 1.8 ml of extraction buffer is added and the powder is suspended by gentle agitation. The tubes are incubated at room temperature for 2-3 days, or at 33°C for 18 h or more. Agitation by hand once or twice during the extraction seems to improve the yield. The beads and cell residue are removed by centrifugation for 15 min at 5000 x G. The supernatants are treated with 5 ml of cold 100% ethanol. The precipitates are'recovered and dispersed in 750 µl of low-salt buffer. Then 4.5 ml of ethanolic perchlorate reagent at room temperature is added to each, and the tubes are put at 4°C for 0.5 h or more. The precipitates are recovered and dissolved in 750 µl of low-salt buffer, and 375 µl of high-salt buffer containing 300 µg/ml of pre-boiled RNAse A is added. The preparations are incubated 1 h at 37°C. They are then mixed thoroughly with equal volume of $CHCl_3$ - isoamyl alcohol (24:1, v/v), and centrifuged. The upper phase from each is transferred to a clean tube, and 2-2.5 volumes of ethanol are added. After 0.5 h or more at 4°C, the tubes are centrifuged, drained well, and the ethanol mostly removed under light vacuum. The pellets are dissolved in about 200 µl of low salt buffer, if rapid solution of DNA is desired, or high-salt buffer for long-term protection against degradation at 4°C. Sometimes one additional precipitation with ethanol results in a preparation that is less contaminated with oligoribonucleotides.

The DNA prepared by this method is readily cleaved by a variety of restriction endonucleases and appears to be at least 25 kilobase pairs or larger. DNA of at least equal molecular weight can be made in about 5-10% yield directly from freshly-harvested mycelial pads simply by dispersing the damp-dry pads in extraction buffer and proceeding as described. We obtained DNA of similar quality from yeast and the Schizophyllum commune, -though for unknown reasons, no DNA was obtained from either of two strains of Aspergillus nidulans. - - - - Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706.