Brief comments on heterocaryosis and crossing methods

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Abstract
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This procedure is the one currently used in our laboratory for the isolation of Neurospora DNA. We have found that several procedures can be applied to Neurospora, but only to liquid nitrogen mycelial powders. However, we prefer this procedure since it consistently yields clean DNA preparations of very high molecular weight.

From a conidial inoculum, Neurospora mycelium are grown in Fernbuch flasks on a rotary shaker table or in bubble cultures. 24 to 72-hour cultures are filtered through a 10 cm. sintered metal funnel, forming thin Neurospora "pancakes". These are cut into small pieces, frozen in liquid nitrogen and ground to a powder in a motor-driven mortar and pestle with the continuous addition of liquid nitrogen. The mycelial powder may then either be used at once or stored at -20°C.

Successful preparation of Neurospora DNA is dependent both upon grinding in liquid nitrogen and upon the detergent sodium dodecyl sulfate (Matheson). Steps subsequent to the liquid nitrogen grinding are essentially a modification of Marmur (1961 J. Mol. Biol. 3:208).

Convenient proportions to use are as follows: 30 gms. of mycelial powder are suspended in 60 ml. of saline-EDTA (NaCl 0.15M, EDTA 0.015M at pH 8) to which is added 6 ml. of 25% sodium dodecyl sulfate. After a 10 min. incubation at 60°C, 12 ml. of 5M sodium perchlorate are added, followed by 80 ml. of chloroform-isoamyl alcohol (24:1, v/v). This mixture is then shaken for 10-15 min. at 0-4°C and all subsequent steps are carried out at this temperature. Centrifugation for about 6 min. at 3,000-7,000 x g results in the formation of three distinct layers; an upper aqueous nucleoprotein phase, a middle layer of denatured protein, and a lower phase of chloroform. The aqueous phase is removed, two volumes of 95% ethanol at -20°C are layered over it, and the nucleoprotein is wound out on a glass rod. After dissolving the nucleoprotein in saline-citrate (NaCl 0.15M, NaCitrate 0.015M at pH 7) it is deproteinized by a 10 min. shaking with an equal volume of chloroform-isoamyl alcohol (Sevag 1938 J. Biol. Chem. 124:425). Centrifugation at 1,600 x g facilitates separation of DNA from denatured protein and chloroform. The upper phase is removed, two volumes ethanol added, DNA wound out, and dissolved in saline-citrate. Three or four more such deproteinizations and ethanol precipitations are usually carried out. To remove contaminating RNA from the preparation, the DNA is wound out in ethanol, redissolved in 0.1M phosphate buffer at pH 7, and RNAase (Worthington) is added so its concentration is 10 μg/ml. After 4 hours incubation at 37°C, the DNA preparation is further deproteinized until a protein layer is no longer found at the interface of the two phases after centrifugation. Finally, the DNA is wound out after overlaying with 0.54 volumes of isoamyl alcohol, the DNA is redissolved in saline-citrate and is stored over chloroform at 0-4°C.

An average S20w of 31 (range 28.8-33.6) has been obtained by ultracentrifugation sedimentation analysis on this DNA. Applying an empirical equation (Burgi and Hershey 1963 Biophys. J. 3:309) to this S20w yields a molecular weight of 26 x 10⁶.

Base ratio analysis by thermal denaturation (Marmur and Doty 1962 J. Mol. Biol. 5:109) shows the DNA to have a 50.4% GC content (personal communication, P. C. Huang). Data from E260/E280 at pH 3 (Frederic, Oth and Fontaine 1961 J. Mol. Biol. 3:11) directly supports this base ratio.

Electron micrographs of such DNA preparations are available upon request.

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the use of tests done in 4" test tubes closed with Oxoid Caps. Both are described in Catcheside 1960 Proc. Roy. Soc. London B153:179; more detail of the plate tests is given in Ahmad and Catcheside 1960 Heredity 15:55. For the tests in tubes we use baskets holding 64 tubes in 8 rows of 8 each. Each basket is labelled in a standard fashion with a code number corresponding to the protocol of the matrix to be set up. Drops of conidial suspension are added to each by means of Pasteur pipettes. The medium contains agar, not sloped, so that the conidial mixture sits on the top and is easily visible on inspection. Daily records are kept on record sheets, for up to 10 to 14 days. Beyond this time the medium tends to dry out too much, so concentrating the constituents. The concentration of the medium, as well as other factors such as the temperature of incubation, affects the ability to grow.

Crossing methods. We use the standard Westergaard and Mitchell formula in 6" tubes. A piece of folded filter paper is inserted into the medium, which contains agar and is sloped. The female parent is allowed to grow first and when abundant protoperithecia are seen to have been formed, the conidial parent is added. For conidiation, quite dense suspensions of conidia are made in 2 ml. of sterile distilled water and this suspension is then added to the slopes containing the female parent, after clearing out any excessive conidial growth that there may be. The tube is rotated between the hands to distribute the conidia and, after a time, the excess liquid is decanted. - - - Department of Genetics, John Curtin School of Medical Research, Australian National University, Canberra, A. C. T., Australia.

Fonkhauser, D. B. Grinding of lyophilized mycelial pads. The grinding with mortar and pestle of lyophilized mycelial pads grown in 125 ml flasks (containing 50 ml of medium) has proven very difficult and time consuming. A much quicker and more effective method is as follows: The lyophilized pad is placed in an 18 x 150 mm test tube and 'chopped' into small pieces with two stainless steel spatulas (8" long with a flat end 2" x 1/4" ). With the spatulas still in the tube, and with the top of the tube held firmly, it is placed inside the cup of a Vortex Jr. Mixer and agitated for 15 to 20 seconds, giving a semi-fine to fine powder according to the length of agitation. A tube will occasionally chip at the top, but this can be minimized by checking for cracked tubes beforehand, and by holding the tube at the top. 20 x 150 mm tubes should not be used because they break too easily. The tube should not be pressed down with any more force than is necessary to hold it in the cup because contact with the screw at the cup base will scratch, and eventually break, the bottom of the tube. In three months, we have never had a tube disintegrate.

This method will grind up to 400 mg of powder, yielding as fine a powder as desired. The enzymes, tryptophan synthetase and indole glycerol phosphate synthetase, are extracted as completely from these powders as from those prepared by use of mortar and pestle. - - - Department of Microbiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio.

Kilbey, B. J. The detection of irreparable mutants in Neurospora. The heterokaryon system used by Atwood and Mukai (1953 Proc. Natl. Acad. Sci. U. S. 39:1027) for the detection of irreparable mutants in Neurospora is open to two main criticisms: first, the heterokaryotic component in which irreparable mutants are scored carries the amycel and methionineless genetic markers, and, second, the tests for reparable are made with medium containing sorbose. Both the genetic background and the plating environment are probably unfavorable for the detection of reparable mutants (Horowitz 1963 NN#3:5).

In an attempt to obviate these criticisms, an entirely new heterokaryon has been prepared. Both the components of the heterokaryon have been derived from the K3/17 strain of Kölmark (Kölmark and Kilbey 1962 Zeit. für Vererbungslehre 93:356). This strain carries a complex of colonial determinants and requires adenine and inositol for growth. The components of the heterokaryon are: