Brandt, E. E. and A. G. DeBusk. Isolation of DNA from Neurospora.

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 mycelia are grown in Fernbach 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 0.01SM 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 overlayering 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 S°_{20w} 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 S°_{20w} yields a molecular weight of 26×10^6.

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 E_{260}/E_{280} 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.

This work was supported in part by a contract (AT-(40-1)-2690) with the Division of Biology and Medicine, U. S. Atomic Energy Commission and the Institute of Molecular Biophysics, Florida State University. - - - Genetics Laboratories, Department of Biological Sciences, Florida State University, Tallahassee, Florida.