Barnett, W. Edgar, C. J. Wust, A. Gib DeBusk and D. Frazier. Isolation of high molecular weight DNA from Neurospora.

The following procedure has been developed to isolate DNA with a minimal molecular weight of 5×10^6 (from ultracentrifugal sedimentation analysis) from *Neurospora crassa*.

1. Mycelia from cultures of N. crassa in early log phase are harvested, washed with distilled water, pressed dry and frozen in liquid nitrogen. The material is ground to a powder under liquid nitrogen in a mortar and pestle. Subsequent steps are carried out at 0–4°C.

2. The mycelial powder is suspended in 0.25 volumes of 0.1 M NaCl buffered at pH 7.75 with 0.1 M Tris and stirred for 10 minutes. Five volumes of ethanol–ether (1:1) are added and stirred for 20 minutes.

3. The suspension is centrifuged at 1000 X g and the supernatant discarded. The pellet is suspended in 0.1 M Tris at pH 7.75 and an equal volume of 5% aerosol OT (Fisher Scientific Co.) is added and stirred either overnight at 0–4°C or 2 hours at room temperature. This suspension is centrifuged at 11,000 X g for 20 minutes and the pellet discarded.

4. NaCl is added to the supernatant to a final concentration of 1 M and isopropanol (at -20°C) is added slowly while the DNA is wound onto a glass rod.

DNA thus isolated may be deproteinized by repeated treatment with 0.05 volumes of chloroform-octanol (8:1) and successive centrifugation to separate the two phases. The procedure is repeated at least 5 times or until there is no interphase (denatured protein). The aqueous phase is made to 1M NaCl and the DNA precipitated with 2 volumes of cold ethanol. The precipitate is dissolved in 0.1 M NaCl and dialyzed.

This procedure is a modification of the method of Astrachan and Volkin (J. Am. Chem. Soc. 79: 130–134, 1957). ---Biology Division, Oak Ridge National Laboratory, [1]Oak Ridge, Tennessee and Florida State University, Tallahassee, Florida, U.S.A. [1]Operated by Union Carbide Corporation for the United States Atomic Energy Commission.