

Isolation of high molecular weight DNA from Neurospora

W. Edgar Barnett

C. j. Wust

A. Gib DeBusk

D. Frazier

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Recommended Citation

Barnett, W. E., C. j. Wust, A. G. DeBusk, and D. Frazier (1963) "Isolation of high molecular weight DNA from Neurospora," *Fungal Genetics Reports*: Vol. 3, Article 5. <https://doi.org/10.4148/1941-4765.2147>

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Abstract

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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 $0-4^{\circ}\text{C}$.

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 $1000 \times g$ 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 $0-4^{\circ}\text{C}$ or 2 hours at room temperature. This suspension is centrifuged at $11,000 \times g$ 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, Oak Ridge, Tennessee and Florida State University, Tallahassee, Florida, U.S.A. 'Operated by Union Carbide Corporation for the United States Atomic Energy Commission.