Dutta, S. K. The irolotion of nuclei from Neurospora crassa conidio.

A greater degree of purity of nuclei isolated from conidiol cells of N. crassa is obtained by combining the procedures described by Reich and Tsuda (1961 Biochem. Biophys. Acta 53:574) and Munkres et al. (1966 Neurospora Newsl. 9: 14) for mycelial cells with some modifications mode in our laboratory. The strain used was wild type 74A and all operations ore conducted in the cold at 0-4°C. The conidiol mass is squeezed dry between Whatman blotting papers and then ground gently with twice its volume of acid-washed sea sand (prepared by powdering the commercially obtained sea sand in a Wiley Mill and parsing through a 60-mesh screen) until a smooth paste is obtained. About five volumes of sucrose-EDTA (0.5M sucrose, 1mM NaEDTA, 0.01M Tris HCl, pH 6.5) is added gradually, stirred into a thick paste, and filtered through four layers of silk cloth. The filtrate is centrifuged at 2000 x g for 25 minutes in a refrigerated centrifuge. This crude nuclear pellet is then suspended in a solution containing 0.5 M sucrose, 2 mM EDTA, and 5 mM $CaCl_2$ at pH 6.5 and centrifuged at low speed (500 x g) for two minutes. Two kinds of pellet were noticed. A hard pellet was formed below the loose pellet. The nuclei contained in the loose pellet were dispersed in their own supernatant and centrifuged again at 500 x g for two minutes. This law-speed centrifugation was repeated until no more hard pellet was noticed.

Comparatively pure nuclear pellet was obtained by parsing this final loose pellet through 1.70 M sucrose solution containing 1 mM EDTA. This nuclear pellet was further cleaned from any cytoplasmic attachments by suspending and stirring for two hours in 10 volumes of saline EDTA (0.08 M NaCl, 0.02 M NaEDTA, pH 6.2). sedimenting at 2000 x g and resuspending in fresh solution of saline EDTA. In our process of chromatin isolation from these nuclei, we use triton-X-100 (0.01 %) along with saline EDTA in order to reduce the surface tension of nuclear membroner. The yield of nuclei was low by this process but a consistent purity (as judged by electron microscopy) was obtained, showing a 5:1 (total protein: DNA) ratio. Supported bv NSF Grant No. Gy3894. ■ ■ ■ Deportment of Botany, Howard University, Washington, D. C. 20001.