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Technical notes on the isolation of Neurospora nuclei

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Technical notes on the isolation of Neurospora nuclei

Abstract

Isolation of Neurospora nuclei

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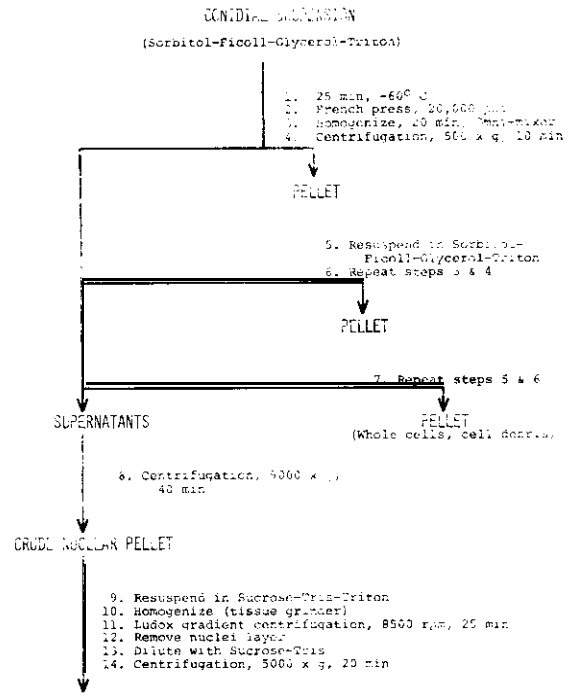
We have recently reported a procedure for isolating Neurospora nuclei from germinated and ungerminated conidia (Hautala, Conner, Jacobson, Patel and Giles 1977 J. Bact. 130:(in press, May). We report here some additional technical notes and suggestions which we hope will assist those utilizing this technique.

The steps in the nuclei isolation procedure are shown schematically in Fig. 1. Complete and efficient freezing during step 1 is essential for successful cell lysis. The French pressure cell should be totally immersed in a dry ice-ethanol bath maintained at -60° C for the entire 25 min period. The French pressure cell should not be completely filled prior to freezing as expansion will force the plunger up to the point that the cell will not fit in a standard French pressure cell press. The optimal procedure is to place 37 ml of suspension in the press with the plunger set at the 40 ml level. The frozen slurry obtained after passage through the French pressure cell (Step 2) is thawed just to the point that no solid remains, diluted immediately, and homogenized with an Omni-Mixer (Step 3) to release the nuclei from the cellular debris. The 200-ml chamber should be used for this step as the release of nuclei is much less efficient in the 400-ml chamber. Inclusion of 0.5% Triton X-100 in the buffer is essential for efficient release of the nuclei during homogenization. Since the pellet obtained in Step 4 is loosely packed, the supernatant should be carefully and immediately withdrawn. Only ca. 70% of the supernatant can be removed without resuspending the pellet which still contains a significant number of free nuclei. Therefore the homogenization and centrifugation steps should be repeated with additional lysis buffer (Steps 5-7) in order to obtain a reasonable nuclear yield.

Figure 1. Isolation scheme for Neurospora nuclei.



The supernatant should be decanted immediately from the crude nuclear pellet (Step 8) to avoid significant resuspension of the nuclei. The crude nuclear pellet should then be resuspended vigorously in the sucrose-Tris-Triton buffer. The presence of 1% Triton in this buffer is essential for removing cytoplasmic material attached to the nuclear membrane. (Step 9). A convenient resuspension method is to rapidly and repeatedly force the solution in and out of a syringe equipped with a 14 gauge cannula. The suspension is then homogenized briefly using a motor driven Potter-Elvehjem tissue grinder (Step 10). The complete homogeneity of the suspension after these manipulations is critical for the success of the subsequent Ludox gradient centrifugation. Once the crude nuclear pellet has been resuspended, it should be subjected immediately to Ludox gradient centrifugation or significant nuclear lysis will occur. Therefore if the sample size requires more than one Ludox gradient centrifugation run, the crude nuclear pellet should be stored on ice and resuspended in batches immediately prior to centrifugation.

Preparation of the Ludox gradients (Step 11) should be done with care as Ludox (a colloidal silica) readily precipitates at low temperatures and on contact with Triton X-100. The 50, 25, and 12.5% Ludox HS-40 solutions are prepared and the step gradient formed at room temperature using the sucrose-Tris buffer. The gradients are then stored at 4° C for 30 to 45 min. The resuspended crude nuclear fraction should be carefully layered on the gradient as any significant mixing at the interface will result in Ludox precipitation due to contact with Triton X-100. We perform the Ludox gradient centrifugation using the SW 27 rotor. However, since the centrifugation speed is only 8500 rpm, this step may be adaptable to the Sorvall preparative swinging bucket rotor. After centrifugation, the nuclei are contained in a discrete, opaque band at the interface of the 50 and 25% Ludox layers. This nuclei layer is removed with a syringe equipped with a cannula and diluted with sucrose-Tris buffer prior to the final centrifugation (Step 14). This dilution lowers the Ludox concentration and thus reduces the chance of contamination of the final nuclear pellet with precipitated Ludox.

Isolating the nuclei in a minimum amount of time is critical as storage of the sample at any intermediate stage results in significant nuclear lysis. We find the optimal experimental protocol to be the processing of 20 g (wet weight) of conidia in an 8 hr period. This involves lysis in the French pressure cell of two 37 ml samples of the conidial suspension. The lysed cell suspension is diluted to ca. 300 ml which is the optimum volume for Omni-Mixer homogenization in the 200-ml chamber. One half of the crude nuclear pellet is resuspended and homogenized in 40 ml of sucrose-Tris-Triton buffer. Six milliters of this suspension is layered onto each of six Ludox gradients. While the first set of gradients is centrifuging, the remainder of the crude nuclear pellet is prepared in another 40 ml of buffer and layered onto another six gradients. The final centrifugation (Step 14) is performed immediately after collecting the nuclei from each set of gradients.

One additional point is that the DNA strain (4,6-diamidino-2-phenyl indole) used to monitor the nuclei isolation is now available from Accurate Chemical and Scientific Corp. in Hicksville, N.Y. (This research was supported by Research Contract E (38-1)-735 with the U.S. Energy Research and Development Administration and Research Grants GM 22054 and GM 23051 from the U.S. Public Health Service. JAH was supported by Public Health Service Postdoctoral Fellowship 5 FC2 GM 55828.) - - - Genetics Program and Department of Zoology, University of Georgia, Athens, GA 30602.