A convenient method for the isolation of crude nuclear pellets

R. Krumlauf
Ohio State University

G. A. Marzluf
Ohio State University

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Abstract
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Figure I. Cultures were harvested and incubated, for six hours, total activity taken up i.e., average of three repeats ± Standard Deviation, total radioactivity incorporated into TCA precipitable counts (♂♀♂).

The desired maternal component strain is inoculated on day 0 into petri plates containing standard Westergaard's crossing medium (Am. J. Bot. (1974) 61: 573-577) supplemented with 2% sucrose and 4% agar.

On the fifth day (or whenever protoperithecia appear) 2 ml of a suspension of conidia of 10^8/ml or greater is pipetted over the plate and spread gently with a spreading bar if necessary. If large amounts of conidia are present on the lid of the plate, there can be removed with tissue wetted with alcohol.

Perithecia can be harvested at any time, by gently scraping the surface with a broad, blunt spatula that has a Burr turned under. This preparation is relatively pure, but additional purification can be obtained by chopping the material in an Omnimixer for twenty seconds at 2,000 rpm. The perithecia are then allowed to settle in a graduated cylinder and collected and concentrated on a filter apparatus. **Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309.**

Johnson, T.E. A method for isolating large quantities of perithecia.


This procedure describes a convenient method for the isolation of crude nuclear pellets from N. crassa. The method, an adaptation of the one developed by H Futalo et al. (1977) J. Bact. 130:704-713, utilizes Braun Homogenizer to disrupt cells. The main advantages of the technique are that the cells need not be frozen, large amounts of material can be handled, the homogenization is fast and easily controlled, fewer omni-mix steps and shorter times are required to release the nuclei, yields are comparable (75%) to those obtained using the French pressure cell, and lower concentrations of ficoll will stabilize the nuclei. The crude nuclear pellets are used to prepare DNA and pure nuclei.

Germinated conidia (14 hrs) are harvested by filtration and rinsed. The Braun Homogenizer disrupts cells via high speed shaking (4000 rpm) with glass beads. Typically, 90 g wet weight of cells are used in each isolation. The 90 g are distributed among four 70 ml glass homogenizer bottles. Each bottle contains 50 g acid washed glass beads (45-100 mm), 10-15 g cells and 11 ml of isolation buffer.

A (Hautala et al., 1977). The isolation buffer, however, contains only 5% Ficoll 400. The cells are kept cold during the homogenization by a jacket fed with siphoned CO2. The cells are homogenized in 30 sec pulses followed by 30 sec rests. Table I shows that optimum yield without

### Table I: Efficiency of Cell Disruption with the Braun Homogenizer

<table>
<thead>
<tr>
<th>time (sec) of homogenization</th>
<th>crude nuclear pellet</th>
<th>membrane pellet</th>
<th>supernatant</th>
<th>crude nuclear supernatant</th>
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<tr>
<td>0</td>
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</tr>
<tr>
<td>120</td>
<td>73</td>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>150</td>
<td>57</td>
<td>6</td>
<td>27</td>
<td>27</td>
</tr>
</tbody>
</table>

Homogenizations were performed in 30 sec pulses followed by 30 sec rests.
lysis nuclei are obtained using 90 sec total homogenization time. The yield at 120 sec is the same but 20% of the nuclei have lysed.

The homogenates plus beads from the four bottler are combined in a beaker and allowed to settle for two minutes. The homogenate is then decanted from the beads. The beads are rinsed three times at 10 minutes with 50 ml of isolation buffer and are saved for RNA. The homogenate and rinses are combined and their volume adjusted to 300 ml. The mixture is then mini-mixed for 10-15 minutes at 4°C. The solution is then centrifuged at 700 xg in a large plastic centrifuge bottle for 10 minutes. The beaded supernatant is saved. The pelleted is resuspended with a syringe in isolation buffer, the volume adjusted to 300 ml and mini-mixed a second time using the same conditions. The solution is centrifuged and the second spin is combined with the first in a large beaker. The crude nucleopellet is obtained by centrifuging the combined supernatants of 9000 xg for 50 minutes. We routinely obtain yields of 65-75% bore on DNA content using this method. See Table II.

The entire procedure requires about four hours. It is possible to handle 180 g of cells by running two homogenizations. While the first homogenate is mini-mixed and centrifuging, the second homogenate is started in the mm-mixer. By overlapping the centrifuge and mini-mix times in this manner and combining all the supernatants to spin down the crude nucleopellet we can handle 180 g in four hours and 360 g conveniently in one day. (Supported by Grant GM-23367 from the National Institutes of Health).

Selitrennikoff, C. P.

Storage of slime strains.

The slime variant of N. crassa (FGSC 4326, fgs256, cr-1, cr-1, cr-1, Arg-1, Arg-1, Arg-1, Arg-1, Arg-1) can be maintained by repeated passage on liquid or agar-solidified medium and can be stored frozen in 10% dimethyl sulfoxide (DMSO) (Creighton and Trevithick (1973) Neurospora News 20: 32) or as a component of a heterokaryon (Nelson et al. (1975) Neurospora News 22: 15-16). However, I have found that petri dishes and culture slants of slime strains can be frozen in situ, stored at -70°C and subsequently thawed and revived. Simply, petri dishes and/or slants containing Nelson's medium B 17.5% sorbitol, 1.5% glucose, 1% agar (and appropriate supplements) are incubated and then frozen for 5-10 days at 28°C. Petri dishes and slants are allowed to thaw completely at room temperature and cell masses transferred to fresh, petri dishes (or slants) with the aid of a rubber policeman. Alternatively, the dishes or slants can be flooded with medium B. The liquid is then pipetted out and the dishes or slants allowed to dry. The plates are then incubated at 10°C for 5-10 days. The organisms grow on the surface of the plates and are readily visible.


The method used to label N. crassa DNA in vitro hybridization reactions is a modification of the technique used by Maniatis et al. (1975, P.N.A.S. 72:1184-1188) to label lambda DNA. Using the ability of EcoRI polymerase I to translate nicks, we artifically nicked DNA with EcoRI and used polymerase I and 3H-dTTP to label DNA, with this technique approximately 26% of the DNA is labeled. The size of the labeled DNA is 247 bases. The DNA is up to 400 hours in culture and is thus suitable for use in a variety of experiments.

The labeled DNA is then nicked with nuclease S1 and used in hybridization reactions. The DNA is a result of the polymerase displacing DNA strands (instead of hydrolyzing them) and then using the displaced strand as a template. The DNA is labeled in this manner by adding the nicking enzyme and allowing it to act for 30 min. The labeled DNA is then placed in alkaline sucrose gradients. The DNA we have isolated in this manner contains 10-20% of the DNA labeled. (Supported by Grant GM-23367 from the National Institutes of Health) - Department of Biochemistry and Developmental Biology Program, Ohio State University.


Electrophoresis of proteins from a single perithecium can be performed in capillary tubes, according to a modification of Gassbach's procedure (1965, Biochim. Biophys. Acta 107: 180-182). Glass capillary tubing with 5-6 mm outside diameter and 1-4/3 mm inside diameter (Thomas Co.) are cut into 4 cm lengths. The column is coated with electrophoretic gel and procedure employed essentially as described for the standard electrophoretic analysis of perithecial extracts (Nasollah and Srb (1973), Proc. Nat. Acad. Sci. USA 70: 3681-3685).