A convenient method for the isolation of crude nuclear pellets

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Abstract
A convenient method for the isolation of crude nuclear pellets

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Figure 1. 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 (o-o-o).

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 containing 107/ml or greater is pipetted over the plate and spread gently with a sterile bar if necessary. If large amounts of conidia are present on the ridges of the plate, they can be removed with tissue wetted with alcohol.


can be handled, the homogenization is fast and easily controlled, fewer 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 Brown 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 75 ml glass homogenizer bottles. Each bottle contains 50 g acid washed glass beads (4.45-5.50 mm), 10-15 g cells and 11 ml of isolation buffer.

Efficiency of Cell Disruption with the Brown Homogenizer.

<table>
<thead>
<tr>
<th>time (sec) of homogenization</th>
<th>crude nuclear pellet</th>
<th>membrane pellet</th>
<th>crude nuclear supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>60</td>
<td>39</td>
<td>57</td>
<td>4</td>
</tr>
<tr>
<td>90</td>
<td>75</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>120</td>
<td>75</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>150</td>
<td>57</td>
<td>6</td>
<td>27</td>
</tr>
</tbody>
</table>

Homogenizations were performed in 30 sec pulses followed by 30 sec rests.
lysing 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. After centrifuging three at a time with 50 ml of isolation buffer and are saved for 1808. The homogenate and rinses are combined and their volume adjusted to 300 ml. The mixture is then mini-mixed for 10-15 minutes. The solution is then centrifuged at 700 x g in a large plastic bottle for 10 minutes. The decanted supernatant is saved.

The pellet is resuspended with a syringe in isolation buffer, the volume adjusted to 300 ml and mini-mixed. In a second time using the same conditions. The solution is centrifuged and the second spin is combined with the first. DNA, the desired nucleopellet is obtained by centrifuging the combined supernatants of 3000 g for 50 minutes. We routinely obtain yields of 65-75% based on DNA content using this method.

The entire procedure requires about 4 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 may be started in the mm-mixer. By overlapping the centrifuge and mini-mixed 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 a day. (Supported by Grant GM-23367 from the National Institutes of Health).

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Selitrennikoff, C. P.

Storage of slime strains.

The slime variant of N. crassa (FGSC 4926; f229ps-I, arg-1, cr-1, our) can be maintained by repeated passage on media of agar-solidified medium and can be stored frozen in 10% dimethyl sulfoxide (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 stereo cultures of slime strains can be frozen in situ, stored at -70°C, and subsequently thawed and revived. Simply, petri dishes or agar plates containing Nelson's agar (and appropriate supplements) are inoculated and incubated for 5-10 days at 28°C. Petri dishes are wrapped with parafilm or aluminum foil (slants are sealed with parafilm) and placed in a -70°C freezer. To revive strains, dishes and slants are allowed to thaw completely at room temperature, and cell masses transferred to fresh dishes (or slants) with the aid of a rubber policeman. Alternatively, cells are allowed to take up medium at room temperature and cell masses transferred to fresh agar-solidified medium. The cells from this medium are transferred to fresh agar-solidified medium.


The method used to label N. crassa DNA in vitro in hybridization reactions is a modification of the technique of Maniatis et al. (1975, P.N.A.S. 72: 1184-1188) to label lambda DNA. Using the ability of E. coli DNA polymerase I to translate nicks, we artifically nicked DNA with DNase I and used powered polymerase I and 32P-dCTP to label DNA, with which this nicked DNA was used as an inoculum for fresh agar-solidified medium. The DNA has been stored for four months and all cultures subsequently revived. Longer storage periods are currently being tested. = = = = Department of Bacteriology, Microbiol, and b., Ins. Rahway, New Jersey 07065.


In this manner we have labeled Neurospora DNA to 3.3 x 107 cpm/ug. DNA activities may be obtained by using more label and other borers may be made this way.

The reaction buffer contains 50 M Tris-HCl, pH 7.8, 1 µg DNA, 5 mM MgCl2, 10 mM β-mercaptoethanol, 50 µg/ml BSA, 5 mM cold dCTPs, 2.5 µM dATP containing 0.75 mol/M, 3H-dATP. The reaction is started by adding 1 x 107 cpm DNase I. The mixture is incubated one minute with the DNA and then for 15 minutes with DNA polymerase I. The reaction is stopped by adding 3 ml of 0.3 M phosphate buffer. 135 M NaCl and 1 % SDS, then boiling for 10 minutes. At this point the reaction mixture contains unincorporated 3H-dATP, labeled DNA and a folded back DNA. The folded back DNA is a result of the DNA and unincorporated material may be removed using hydroxyapatite. The folded back DNA may be separated from the DNA and labeled DNA which is not a result of the reaction. The labeled DNA may be eluted with 5 mM NaCl and the DNA labeled DNA should then be sedimented on an alkaline sucrose gradient. The DNA we have labeled and have been isolated in this manner contains very little folded back DNA and represents 75% of the DNA labeled.

(20x13)

Nasrallah, J.B. and A.M. Srbr. Microelectrophoresis of extracts from single perithecia. Microelectrophoresis of proteins from a single peritheciun can be performed in capillary tubes, according to modification of Grobstein's procedure (1965, Biochim. Biophys. Acta 107: 180-182). Glass capillary tubing with 5-6 mm outside diameter and 1/4-3/4 mm inside diameter (Thomas Co.) are cut into 4 cm lengths. The Column Coot electrophoretic system and procedure employed are essentially as described for the standard electrophoretic analysis of perithecal extracts (Nasrallah and Srbr (1973), Proc. Nat. Acad. Sci. USA 70: