Cultures were harvested and incubated, for six hours, total activity taken up is,
overage of three repeats ± Standard Deviation,
total radioactivity incorporated into TCA precip-
itble counts (o-o-o).

Figure 1. Cultures were harvested and incubated, for six hours, total activity taken up is,
overage of three repeats ± Standard Deviation,
total radioactivity incorporated into TCA precip-
itble counts (o-o-o).

Then ground (Narasallah and Sriv 1973) Proc. No. Acad. Sci. USA 70: 1891-1893, centrifuged at 5,000 g for 5 minutes and the supernatant frozen at -60°C. Before use, the sample is thawed and centrifuged again at 5,000 g for 5 minutes.

The amount of label incorporated into TCA precipitable material plateaus at about four hours. Labeled proteins can be obtained at any point in development by using this technique; however, a drop-off both in uptake and in incorporation at later points in the development is seen (Figure 1). Using 35S-methionine (2.2 μCi, 700mCi/mmol), I have obtained extracts containing as much as 1000 cpm/μg protein. This technique provides a very efficient uptake of label with minimum handling after labeling.

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309.

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

spread by necessary, if large amounts of conditions are not present on 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 5,000 rpm. The perithecia are then allowed to settle out in a graduated cylinder and collected and concentrated on a filter apparatus. • • Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309.


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 Hautala 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 dispersed among four 75 ml glass homogenizer bottles. Each bottle contains 50 g acid washed glass beads (45-50 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 homogenization by a jacketed fed with superfloated CO2. The cells are homogenized in 30 sec pulses followed by 30 sec rests. Table 1 shows that optimum yields without

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Efficiency of Cell Disruption with the Braun Homogenizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>time (sec) of homogenization</td>
<td>crude nuclear pellet</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>60</td>
<td>39</td>
</tr>
<tr>
<td>120</td>
<td>73</td>
</tr>
<tr>
<td>150</td>
<td>57</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. The beads are rinsed three at four times with 50 ml of isolation buffer and are saved for 186U. The homogenate and rinses are combined and their volume adjusted to 300 ml. The mixture is then mini-mixed for 15-30 minutes. The solution is then centrifuged at 700 x g in a large plastic centrifuge 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 a second time using the same conditions. The solution is centrifuged and the second spin is combined with the first fraction. The crude preparation is obtained by centrifuging the combined supernatants of 9000 x g 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 100 g of cells by running two homogenizations. While the first homogenate is mini-mixed and centrifuging, the second homogenate may be started in the min-mixer. By overlapping the centrifuge and mini-mix times in this manner and combining the supernatants to spin down the crude nuclear pellet 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).

- - Department of Biochemistry and The Developmental Biology Program, Ohio State University, Columbus, Ohio 43210.

Selittrennikoff, C. P.

Storage of slime strains.

The slime variant of N. crosse (FGSC 4926; f259955-1, arg-1, c-, 1, cur), can be maintained by repeated passage on solidified agar solidified medium and can be stored frozen in 10% dimethyl sulfoxide (Creighton and Trevithick (1979) Neurospora News 20: 32) or as a component of a heterokaryon (Nelson et al. (1979) Neurospora News. 22: 15-16). However, I have found that petri dishes and slant cultures of slime strains can be frozen in situ, stored at 70°C and subsequently thawed and revived. Simple. petri dishes and/or slants containing Nelson's solidified 1.5% agar (appropriate room temperature supplements) are inoculated and incubated at 28°C for 5-10 days. Petri dishes and/or 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, medium B 17.5% sucrose (v/v), 1% Vogel's Salt's solidified with 1.5% agar (and appropriate room temperature supplements) are inoculated and incubated at 28°C for 5-10 days. Petri dishes and/or 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. Medium B is used as an inoculum for fresh, agar-solidified medium. Thus for slime and two derivatives strains (slime strains containing cur-1 on isolates) have been stored for four months and all cultures subsequently revived. Longer storage periods are currently being tested. - - Department of Botic Microbiology, Merck and Co., Inc., Rahway, New Jersey 07065.


The method we have used to label N. crosse DNA in vitro modification reactions is a modification of the technique used by Mariat 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 artificially nicked DNA with DNase I and used polymerase 1 and 3H-dTTP to label DNA, with this technique which represents 26% incorporation of label into DNA. A higher specific activity of base labeled DNA can be obtained by using labeled E. coli DNA as a primer. The labeled DNA is 247 bases.

The reaction buffer contains 50 mM Tris-HCl, pH 7.8, 1.0 µg/ml DNA, 5.0 mM MgCl2, 10.0 mM β-mercaptoethanol, 50 µg/ml BSA, 5.0 µM cold dNTPs and 2.5 µM 3H-dTTP containing 0.5 µCi 3H-dTTP. The reaction mixture is incubated at 15°C for 10 minutes and the reaction is stopped by adding 1 x 10-6 M DNase I. The mixture is incubated one minute with the nuclease when 12.5 units of DNA polymerase I 38.0 relationships. The reaction was repeated after about 8-10 hours, but incorporation continues up to four hours.

The reaction is stopped by adding 3 ml of 0.3 M phosphate buffer. 1.5 M NaCl and 1% SDS, then boiling for 10 minutes. At this point the reaction mixture contains unincorporated 3H-dTTP, labeled DNA and a labeled foldback DNA. The foldback DNA is a result of the polymerase displacing DNA strands (instead of hydrolyzing them) and then using the displaced DNA as a template. The foldback DNA and unincorporated material may both be removed using hydroxyapatite. The reaction mixture is dialyzed against the column of HAP at 50°C. At 50°C only single stranded and double stranded DNA will bind in 0.3 M phosphate buffer. Extensive washing with 0.3 M PB will be eluted the unincorporated material and small fragments. Since the foldback DNA contains double stranded regions it may be separated from the labeled DNA which is almost single stranded (100°C, 5 min) by eluting the later from the column with 1.5 M PB. The foldback DNA may be eluted at the column using 50°C PB.

The labeled DNA should then be aired on an alkaline sucrose gradients. The DNA we have labeled and purified in this manner contains very little foldback DNA and represents 75% of the DNA labeled. (Supported by Grant GM-23367 from the National Institutes of Health).


Electrophoresis of proteins from a single perithecia can be performed in capillary tubes, according to a modification of Cross'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 Coat electrophoresis system and procedure employed essentially as described in the standard electrophoretic analysis of peripheral extracts (Nasrallah and Srbi (1973), Proc. Nat. Acad. Sci. USA 70: 499.)

(Canadco) coated tubes are sealed at one end with parafilm. The electrophoretic system and procedure employed essentially as described in the standard electrophoretic analysis of peripheral extracts (Nasrallah and Srbi (1973), Proc. Nat. Acad. Sci. USA 70: 499.)