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 2 minutes. The homogenate is then decanted from the beads. The beads are rinsed three times at four times with 50 ml of isolation buffer and are saved for 180C. The homogenate and rinses are combined and their volume adjusted to 300 ml. The mixture is then mini-mixed for 10-15 minutes at setting or 0.0. The solution is then centrifuged at 700 x g for 10 minutes in the top centrifuge bottle for 10 minutes. 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. The crude nuclear pellet is obtained by centrifuging the combined supernatants of 9000 x g for 50 minutes. We routinely obtain yields of 65-75% borel on DNA content using this method.

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 may be 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 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). A = = Department of Biochemistry and Developmental Biology Program, Ohio State University, Columbus, Ohio 43210.

Seltenrömkkoff, C. P.

Storage of slime strains.

The slime variant of N. crassa (FGSC #26; f239;ps=1, arg-1, cr-1, cur) can be maintained by repeated passage on liquid 6% 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 dish and slant cultures of slime strains can be frozen in situ, stored at 70 OCC and subsequently thawed and revived. Simply, petri dishes and/or slants containing Nelson's solid medium B 17.5% agar, 1.5% Sucrose (w/v), 1X Vogel's Salt's solution, or solidified with 1.5% agar (and appropriate growth factors) are incubated for 5-10 days at 28°C. Petri dishes are wrapped and stored at -70 OCC. When needed, the dishes and slants are allowed to thaw completely at room temperature and are then transferred to fresh dishes (or slants) with the aid of a rubber policeman. Alternatively, the slime may be transferred to new petri dishes or used as an inoculum for fresh agar-solidified medium. Thus far, slime and two derivatives strains (slime strains containing cur-1 or cr-1) have been stored for four months and all cultures subsequently revived. Longer storage periods are currently being tested. A = Department of Botic Microbiology, Merck and b., Ins., Rahway, New Jersey 07065.


A method for labeling DNA in vitro using nicked translation.

In this technique we have used Neurospora DNA or 3.3 x 10^7 cm/µg and we have labeled Neurospora DNA to 3.3 x 10^7 cm/µg. Enzymatic activities may be obtained by using more label and other techniques may be made this way.

The reaction buffer contains 50 m M Tris.HCl, pH 7.8, 0.1 mg DNA, 5 mM MgCl₂, 10 mM d-mercaptoethanol, 50 µg/ml BSA, 5 µM dNTPs, 2.5 µM dTTP containing 0.5 mCi ³H-dTTP. The mixture is incubated at 150 °CO for 10 minutes and the reaction is stopped by adding 1 x 10⁻⁴ M DNAase I. The mixture is incubated one minute with 25 units of DNA polymerase I (1 unit is defined as the amount of enzyme that will incorporate 1 µg of DNA into DNA NTP/A in 1 hour).

The reaction is stopped by adding 3 ml of 0.1 M NaCl and 1% SDS, then boiling for 10 minutes. At this point the reaction mixture contains unincorporated ³H-dTTP, labeled DNA and DNAase. The DNAase is a result of the polymerase displacing DNA strands (instead of hydrolyzing them) and then using the displaced DNA as a template. The DNAase and unincorporated material may be excised using hydroxyapatite. The DNAase reaction is then added to a column of HAP at 50 °CO for 10 minutes. The DNAase reaction and enzyme are removed by washing with 0.1 M NaCl. The DNA is eluted from the column by 5% NaCl and 20% sucrose.

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