A method for labeling DNA in vitro using nicked translation

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Abstract
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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 1969. The homogenate mixture is centrifuged at 100,000 rpm for 60 min. The supernatant is then centrifuged in a large plastic centrifuge bottle for 10 minutes. The precipitate is resuspended in a syringe in isolation buffer, volume adjusted to 300 ml and mixed with a second time using the same conditions. The solution is centrifuged and the supernatant is combined with the second spin. The crude nuclear pellet is obtained by centrifuging the combined supernatants of 9000 rpm for 10 minutes. We routinely obtain yields of 65-75% bore 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 mixed and centrifuging, the second homogenate is cenfuged in the mm-mixer. By overlapping the centrifuge and mixing 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 one day. (Supported by Grant GM-23367 from the National Institutes of Health.)

Selitrennikoff, C. P.

Storage of slime strains.

Krumlauf, R. and C. A. Marzluf

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TABLE II

<table>
<thead>
<tr>
<th>Yield Comparisons of DNA Using Different Techniques</th>
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<td>homogenization</td>
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<td>1. french pressure cell</td>
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DNA concentrations were measured by the dienphyleinamine test (Gilles et al., 1965, Nature 206: 93).

The slime variant of N. crassa (FGSC 9326, f.2ssagp-1, arg-l, cr-l, our) can be maintained by repeated passage on either 6-agar solidified medium and can be stored frozen in 10% dimethyl sulfoxide (Creighton and Trevithick, 1973) Neurospora News 20: 32) as a component of a heterokaryon (Nelson et al., 1975) Neurospora News 22: 15-16). However, I have found that petri dish and slants cultures of slime strains can be stored in situ, stored at 70°C and subsequently thawed and revived. Simply, petri dishes and/or slants containing Nelson's solidified agar (ond appropriate supplements) are inoculated and incubated for 3-10 days at 28°C. To revive, dishes and/or slants are treated with water and cultured at room temperature on fresh media. Cells suspended or slants are passed on fresh agar and cultured at room temperature for 3-10 days. Cultures are transferred to fresh agar dishes or slants with the aid of a rubber policeman. Alternatively, plates are wrapped in aluminum foil (slants are sealed with parafilm) and placed in a -70°C freezer. To revive, dishes and/or slants are thawed completely at room temperature and cell masses transferred to fresh agar dishes or slants with the aid of a rubber policeman. This procedure and conditions of N. crassa (FGSC 9326) have been stored for four months and all cultures subsequently revived. Longer storage periods are currently being tested.

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