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A convenient method for the isolation of crude nuclear pellets

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Abstract

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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 distributed 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 the homogenization by a jacket fed with siphoned CO_2. The cells are homogenized in 30 sec pulses followed by 30 sec rests. Table I shows that optimum yields without

TABLE I

Efficiency of Cell Disruption with the Braun Homogenizer

time (sec) of homogenization	% yield and distribution of DNA		
	crude nuclear pellet	membrane pellet	crude nuclear supernatant
0	0	100	0
30	25	70	5
60	39	57	4
90	75	20	5
120	73	8	19
150	57	6	37

Homogenizations were performed in 30 sec pluses 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 or four timer with 50 ml of isolation buffer and are saved for reuse. The homegenate and rinses are combined and their volume adjusted to 300 ml. The mixture is then mni-mixed for 10-15 minutes ❑ t a setting of 6.0. The solution is then centrifuged at 700 xg in large plastic centrifuge bottler for 10 minutes. The decanted supernatant is roved. The pellet is resuspended with a syringe in isolation buffer, the volume adjusted to 300 ml and omni-mixed a second time using the same conditions. The solution is centrifuged and the second spin is combined with the first supernatant. The crude nuclear pellet is obtained by centrifuging the combined supernatants at 9000 xg for 50 minute.. We routinely obtain yields of 65-75% bored on DNA content using this method. be Table II.

TABLE II

Yield Comparisons of DNA Using Different Techniques

method of homogenization	% yields bored on whole cells	crud	DNA	pure nuclei
1 french pressure cell cell frozen	100	70-80		25
2. french pressure cell cell cold	100	65-72		22
3 . Braun homogenizer 90 sec	100	65-75		26
4. hand shaking with glass beads 10 min	100	20		2-3

DNA concentrations were measured by the diphenylamine method (Giles et al. 1965, Nature 206: 93).

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 omni-mixed and centrifuging, the second homogenate may be started in the mm-mixer. By overlapping the centrifuge and omni-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).

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