An efficient isolation method for meiotic mutants causing meiotic nondisjunction or elevated recombination frequency in Neurospora crassa.

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**Abstract**
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will themselves be disrupted. Because large batches require longer homogenization for comparable breakage, we recommend processing successive, smaller lots of mycelium for organelle recovery.

The bead-beater may be used for whole-cell extracts if large amounts of mycelia need to be homogenized extensively. These preparations may be somewhat dilute; bead-free, broken cell extracts have an approximate volume of 15-, 40-, and 250-ml from the three chambers, respectively. However, ammonium sulfate precipitation can be applied to the extract, allowing soluble proteins to be concentrated by centrifugation.

The bead-beater is available from Biospec Products, P.O. Box 722, Bartlesville, OK 74003 for about $400. The company also sells the glass beads. It is also available from Tekmar, P.O. Box 37202, Cincinnati, OH 4522, (800) 543-4461. Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, CA 92717.

Hasunuma, K. and K. Furukawa
An efficient isolation method for meiotic mutants causing meiotic nondisjunction or elevated recombination frequency in Neurospora crassa.

Among several inositol-requiring mutants, inl (37401 and inl (83201(t)) complement to each other. This fact gave us an antiselection method of aneuploid ascospores in linkage group V, which contain meiotic mutants causing chromosomal nondisjunction. Ascospores haploid in linkage group V will be led to inositol-less death on minimal plates at 37°C. Meiotic mutants which cause elevated recombination frequencies can also be selected by this method. Conidia from strains K1204a (lys-1 (33933)), inl (37401, his-6 (Y152M105; nuc-2 (T28M2)) and 2309A (al-3 (RP100), in1 (83201(t))) were irradiated with UV for 0, 30 and 60 sec (10% survival) and intercrossed. The resulting ascospores were plated in Fries minimal plates (5.1 x 104 ascospores/plate with non-irradiated sample) and incubated at 37°C for 3 days. From 0, 30 and 60 sec irradiated samples 571, 1100 and 528 of colonies were isolated (40 colonies/plate with non-irradiated sample). Most of the strains isolated had pale orange conidia which are intermediate in color between those of al-3 and wild type. Conidial isolation of these cultures revealed segregation of al-3. They were crossed with strain 2125A and 2998a in 12 x 105 mm test tubes. As shown in Fig. 1, strains discharging more than 20% of abortive ascospores were screened and 60, 167 and 56 strains were obtained from each sample.

From each of these crosses of the 60 strains obtained as spontaneous mutants, 50 or 100 ascospores were isolated. White ascospore production by these isolates was analyzed by crossing with wild type, with the isolates as protoperithecial parents. When more than 30% of isolates showed more than 30% of abortive ascospores, these strains were reserved for further analysis. Isolation of single conidia of appropriate isolates which showed meiotic aberration was performed and those which produced more than 30% of abortive white ascospores when crossed as protoperithecial parents were used for further analysis. This process was essential to avoid heterogenous genetic backgrounds caused by meiotic nondisjunction. These strains thus obtained were reciprocally crossed and resulting ascospores were analyzed. As shown in Table 1, these mutants were grouped into two classes. Class I includes those strains which produced 30 to 90% of abortive white ascospores only when they were crossed as protoperithecial parents. With homozygous crosses, higher frequencies of abortion were observed.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ascospore shot (% of white spore)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Protoperithecia / conidia)</td>
<td>Class I</td>
</tr>
<tr>
<td>+ / +</td>
<td>0-5</td>
</tr>
<tr>
<td>+ / -</td>
<td>0-5</td>
</tr>
<tr>
<td>Mei / +</td>
<td>40-70</td>
</tr>
<tr>
<td>Mei / Mei</td>
<td>90</td>
</tr>
</tbody>
</table>

*Perithecia were formed but no discharge of ascospore was observed.

Table I
White abortive ascospore production in isolated meiotic mutants.

Figure 1. Distribution of percent of white abortive ascospores produced from crosses between nutrition nonrequiring isolates and wild type, 2125A or 2998a. Experimental details were as described in the text. Crossed with 2125A (---) or 2998a (-----). Accumulation of 0, 30, and 60 sec irradiated samples (- - -).
I have been exploring possible applications of fluorescence microscopy to routine Neurospora cytology. I have used five fluorochromes that have specificity for DNA: DAPI (4,6-diamidino-2-phenylindole), Hoechst 33258, olivomycin, auramin-O and acriflavine. DAPI, Hoechst 33258 and olivomycin are simplest and quickest to use. Acriflavine is very good for observations on ascus nuclear cytology. DAPI and Hoechst 33258 require a mercury light source and fluorite objectives. With auramin-O, the staining solution must be prepared fresh every time, and requires filtering. (See Lenke et al. 1975 Exptl. Cell Res. 96: 367.) Acriflavine and auramin-O are very inexpensive and easily available. The other three are more expensive, but their cost is not prohibitive because of the very small quantities needed. Acriflavine- and auramin-O staining procedures are based on Feulgen, and use of acriflavine for quantitative DNA measurements has been reported. Some fluorochromes (DAPI, olivomycin, Hoechst 3342) are also useful as vital nuclear stains. I have used five fluorochromes that have specificity for DNA: DAPI (4,6-diamidino-2-phenylindole), Hoechst 33258, olivomycin, auramin-O and acriflavine. DAPI, Hoechst 33258 and olivomycin are simplest and quickest to use. Acriflavine is very good for observations on ascus nuclear cytology. DAPI and Hoechst 33258 require a mercury light source and fluorite objectives. With auramin-O, the staining solution must be prepared fresh every time, and requires filtering. (See Lenke et al. 1975 Exptl. Cell Res. 96: 367.) Acriflavine and auramin-O are very inexpensive and easily available. The other three are more expensive, but their cost is not prohibitive because of the very small quantities needed. Acriflavine- and auramin-O staining procedures are based on Feulgen, and use of acriflavine for quantitative DNA measurements has been reported. Some fluorochromes (DAPI, olivomycin, Hoechst 3342) are also useful as vital nuclear stains.