

Aiuto, R. A rapid method for obtaining linkage

group IV double mutant stocks.

In a current fine-structure analysis of the methionine-1 locus, a method of obtaining me-1 with the desired right-hand and left-hand outside markers has been developed. This involves the use of the temperature-sensitive colonial mutant cot. The method can be used with any pair of markers proximal or distal to it.

Using the cross me-1 x hist-5, cot as an example, the method is as follows: Ascospores are harvested with a loop of sterile distilled water, suspended in 10 ml of sterile distilled water, heat-shocked for 30 minutes at 60°C, and 0.5 ml of this suspension per plate is spread on several hirtidine-supplemented plates. A firmer medium (3% agar) facilitates isolation. These plates are incubated at 32°C for eight to ten hours. Since the order is me-1 - hist-5 - cot, most of the ascospores that grow will be the hist-5, cot parents and one-half the single crossovers in region 2. These growing ascospores are ignored, and 100 of the germinated but non-growing ascospores are isolated to methionine plus hirtidine-supplemented tubes. These isolates are incubated at 32°C for 48 hours. The cot⁺ isolates (those with wild-type morphology) are discarded, and the cot⁻ isolates are allowed to grow up at 25°C. The latter isolates are then tested on methionine-supplemented liquid medium, and approximately 15% of them will show no growth, indicating that they are the desired me-1, hist-5, cot recombinants.

If one wishes to obtain me-1 with a particular left-hand marker, soy pyridoxine-1, the procedure is reversed. The ascospores are plated on methionine medium, germinated but non-growing ascospores are isolated to methionine plus pyridoxine-supplemented tubes, and the cot⁻ isolates are discarded. In this instance, upon testing on methionine-supplemented liquid medium, approximately 12% of these remaining cot⁺ isolates will show no growth and be the desired pdx-1, me-1 recombinants.

Table 1. Comparison of random ascospore isolations and selection technique in obtaining specifically desired double mutants.

Cross	No. of ascospores isolated	No. of ascospores germinated	No. of isolates tested	Genotype and no. of desired double mutants obtained
<u>me-1 A x his⁺-5, cot a</u>	200 (random)	179	179	3 <u>me-1, hirt-5, cot</u>
	100 (selected)	80	14	3 <u>me-1, hist-5, cot</u>
<u>pdx-1, cot A x me-1 a</u>	100 (random)	98	98	0
	85 (selected)	80	9	1 <u>pdx-1, me-1</u>

This method reduces drastically the amount of manipulation necessary to obtain stocks of double mutants which are closely linked (Table 1), but requires that all stocks being crossed to the mutant of primary interest carry cot. The temperature-sensitive mutants of unknown requirement (un (b39), un (44409), and un (55701)) of linkage group I, and the riboflavin-1 mutant (51602t) of linkage group VI, as well as other temperature-sensitive or pigment-depositing mutants, could be used in a similar fashion. - - - Department of Botany, University of North Carolina, Chapel Hill, North Carolina (present address: Department of Biology, Albion College, Albion, Michigan. 49224).