

A method for obtaining double mutants within single genes or gene clusters

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Abstract

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for genetic mapping studies at many loci in *Neurospora*, as well as in other organisms which form heterocaryons producing multinucleate conidia and in other types such as yeast or *Aspergillus* which produce diploid heterozygous single cells or conidia.

Basically, the procedure in *Neurospora* involves forming a heterocaryon between two complementing mutants within the same cistron or operon with each of the strains carrying a different, unrelated biochemical mutation. Conidia from such a heterocaryon are then treated with an appropriate mutagen, subjected to the filtration concentration procedure on minimal medium and then plated on minimal medium containing only the growth supplement normally required by the single original complementing mutants. Under these conditions, selection will occur for heterocaryotic conidia containing induced double mutants (in either of the two parental nuclei) which now cannot complement with the original single parental type nucleus.

In the studies at the *hist-3* region, two different heterocaryons were used (both mating type A). The first heterocaryon combined a *hist-3A* mutant (M127) carrying an adenine forcing mutant *ad-6* and a *hist-3D* mutant (M234) carrying a niacin forcing mutant *nic-2* (43002). The second heterocaryon involved the same *hist-3A* parent with the *ad-6* mutant and a *hist-3B* mutant (M1352) with the same *nic-2* strain. The double mutants were detected by their inability to grow on minimal medium and were extracted from the heterocaryons either by conidial plating or by outcrossing. The second site mutants in the resulting homocaryotic double mutants were then characterized by their complementation pattern with the tester strains *hist-3A* (M127), *hist-3B* (M1352) and *hist-3D* (M234), by their mapping pattern with the other *hist-3* mutants, and by enzymatic assays for the three reactions in histidine biosynthesis controlled by the *hist-3* region. By using this procedure, a large number of presumptive double *hist-3* mutants were obtained. Many of the double mutants involved lethal mutants which could not be extracted from the heterocaryons either by plating or by outcrossing. Fifteen double mutants were completely characterized. Eight double mutants were recovered in the *hist-3B* strain. Five of these second site mutants were noncomplementing, two were *hist-3A* mutants, and one was a *hist-3D* mutant. Seven double mutants were recovered in the *hist-3D* strain. Six of the second site mutants were noncomplementing, and one was a *hist-3A* mutant. (Supported by AEC contract AT (30-1)-3098.) - - - Department of Biology, Yale University, New Haven, Connecticut 06520.

Double mutants within the *hist-3* region have been obtained by a technique utilizing heterocaryons similar to that described by de Serres and Osterbind (1962 *Genetics* 47:793). This procedure makes possible the recovery of double mutants within single genes (cistrons) or within operon-type systems. This technique should be of general applicability