Fungal Genetics Reports

Volume 10 Article 16

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Recommended Citation

Aiuto, R. (1966) "Intergenic mapping by a sorbose selection technique.," *Fungal Genetics Reports*: Vol. 10, Article 16. https://doi.org/10.4148/1941-4765.2006

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Abstract

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Aiuto, R . Intergenic mapping by a sorbose selection technique.

The following exercise has been found useful in laboratory sections in which adequate numbers of dissecting microscopes are not available for Neurospora ascospore isolation. It is adapted from methods presented by Lavigne (1962 NN# 2:20), Smith (1962 NN# 1:16) and Frost (as described in Fincham and Day 1962 Fungal genetics, 1st ed. Blackwell, Oxford). The technique requires the use of a biochemical mutant linked to the temperature-sensitive colonial mutant cot, crossed to another linkage group IV biochemical mutant, and the use of the growth-inhibiting carbon source, sorbose.

Ascospores from a cross of pdx-1, cot x pan-1 are harvested with a sterile loop and suspended in 5 ml of sterile distilled water. Usually a single loop of ascospores is more than sufficient for mapping purposes. Four differently supplemented flasks of medium N are prepared (2% agar, 1% sorbose, 0.1% sucrose), each containing 50 ml. One ml of the ascospore suspension is heat-shocked at 60C for 30 minutes in each of the flasks of molten medium, and two plates are poured from each flask. After 60 hours of incubation at 33C, two types of colonies can be recognized: typically sorbose-inhibited colonies three tofour mm. in diameter, and colonies about 1 mm. in diameter. These are cot^+ and cot^- individuals, respectively.

The two minimal plates reveal gene order. Most of the colonies will be cot^- and represent one-half the single crossovers in region 1, while the cot^+ colonies, far fewer in number, are one-half the doubles. Colonies scored on pyridoxine-supplemented medium (0.2 mg/ml) as cot^+ are one-half the single crossovers in region 2 less the number of cot+ colonies observed on minimal medium. Colonies scored as cot^- on plates supplemented with calcium pantothenate (0.1 mg/ml) represent the reciprocal single crossover class of region 2 less the number of cot^- colonies observed on minimal medium. Completely supplemented plates, those supplemented with both pyridoxine and caIcium pantothenate, furnish the total number of viable ascospores plated, and the total number in each parental class. This latter calculation is determined by the difference of the recombinant classes (counting the values obtained for singles in region 1 and doubles twice) from the total number of colonies appearing on complete medium.

Other linkage group IV crosses can be used, such as me-1 x his-5, cot. Some mutant combinations, however, e.g., pyr-l, cot, do not lend themselves to clear-cut discrimination of $cot^+$and cot^- colonies, nor do all mutant combinations respond satisfactorily at 33C. In such instances, colonies must be isolated to appropriately supplemented non-sorbose tubes, i.e., from minimal plates to minimal tubes, and scoring is then done with the tubes.

In addition to permitting a mapping exercise with Neurospora without the use of dissecting microscopes, this method introduces the student to a plating technique, and allows instructor and class to discuss the theoretical basis for mapping genes with recombinant classes incompletely identified. The exercise can be shortened by considering only gene order (using only minimal plates) or extended to a four-point cross by isolating colonies to individual tubes, allowing these isolates to grow up at 25C on non-sorbose medium, and testing in liquid medium for the fourth marker. - - - Department of Biology, Albion College, Albion, Michigan 49224.