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Abstract
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In order to adequately evaluate reversion kinetics, the mode of action of mutagens and to determine functional and non-functional regions of the gene, it would be useful to clearly distinguish between true molecular reversions and intra-genic second site suppressor reversions. We have developed a technique based on the phenomenon of inositol-less death (Lester and Gross 1959 Science 129: 572) designed to distinguish between these two types of reversion. This technique permits the selective survival of certain infrequent recombinants with adequate resolution to detect recombination between adjacent nucleotide base pairs.

If a revertant is a result of a second site mutation, recombination between the two sites should yield the original auxotrophic phenotype, and some of the second site mutations isolated by recombination may or may not have mutant phenotype. The difficulty has been in detecting infrequent recombinant types from among a large number of progeny. Our procedure permits the selective survival of recombinant spores which have nutritional requirement in addition to their inositol requirement.

An auxotrophic mutant is either induced or crossed into an inositol requiring strain (8960l). Such a double mutant stock (e.g., inos; arg-10) is subjected to a mutagen and revertants recovered which still require inositol. Such a revertant (inos; arg-10-R) is crossed to an inositol-less stock (with appropriate markers) of the opposite mating type. The resulting spores are subjected to inositol-less death, which permits the selective survival of any recombinants resulting from the separation of a mutant from its suppressor.

Crosses are made on filter paper cones placed in screw-capped flasks with Westergaard's medium, l % glucose, l μM/ml inositol and additional supplements when appropriate. After 30 days, spores are harvested by swirling the liquid medium around the sides of the flasks. The spore suspension is filtered through cheese cloth to remove perithecia and other debris. This spore suspension is filtered on to a millipore filter and resuspended in water. The spores are heat shocked for 60 min. at 60°C and spread (usually 10,000 to 100,000 spores/plate) on petri plates containing Vogel's salts, sorbose and sucrose at a ratio of 15:1. This medium must contain all appropriate supplements except inositol and the requirement of the mutant being tested for a suppressor.

Following 4-6 days of incubation at 30°C, these plates are over-layered with medium containing l μm/ml of inositol and the proper concentration of the requirement for the mutant in question. All resulting colonies are isolated onto slants and screened for the original mutant phenotype and recombination of the marker mutations.

A lys-3 and an arg-10 mutant have been tested in reconstruction experiments. The results of some typical experiments are as follows. When 8 viable inos; lys spores were mixed with 5×10^3 inos spores prototrophic for lys, 7 of 35 colonies surviving inositol-less death had the inos; lys phenotype. When 135 viable inos; lys spores were mixed with 6.5×10^4 inos spores, all 53 surviving spores had the inos; lys phenotype. These experiments represent the extremes in percentage (39-87%) of the recovery of the input inos; lys spore type. With similar numbers of spores, the range in recovery of the input inos; arg-10 spores was found to be 12-24 %. The survival of the lys or arg prototrophic spore types was very small, usually less than 0.01% of the input number.

A UV-induced revertant of the above-mentioned arg-10 (UV) mutant was subjected to this technique. Analysis of 2.3×10^7 viable spores revealed no suppressors. Since about 10^7 spores should yield complete resolution, even assuming as low as 10% recovery efficiency, it is assumed that this UV revertant represents a true molecular reversion and not a suppressor mutation.

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