How to use asci for obtaining double mutants of genes that show epistasis or are phenotypically similar.

David Perkins

Background

Double mutants were used to establish the sequence of gene action in the first biosynthetic pathway. Srb and Horowitz (1944) crossed a Neurospora strain that could use ornithine or citrulline or arginine by a strain that could use citrulline or arginine but not ornithine. The double mutant, obtained from a nonparental ditype ascus (Srb 1946), was able to grow on citrulline or arginine but not on ornithine. That is, the mutant impaired in a later step in the pathway was epistatic to that impaired at an earlier step. The phenotype of one of the mutations cannot be scored in strains carrying the epistatic mutation.

Double mutants have since been used in a variety of ways, such as placing DNA-repair mutants in epistasis groups (Kafer 1983, Inoue 1999), determining epistasis of morphological mutants (Gavric and Griffiths 2003), and determining the temporal order of gene action (Jarvik and Botstein 1973, Moir and Botstein 1982, Gavric and Griffiths 2003). Doubles have also been used to characterize suppressor muttions (e.g., Seale 1976) and to create mutant combinations that would provide screens for novel mutant types (e.g., Davis 1962). In many situations, the phenotype of the double mutant is not known in advance or is difficult to distinguish from the phenotype of a single mutant.

Regardless of the single- and double-mutant phenotypes, double mutants can be obtained by isolating asci for tetrad analysis. When two nonallelic single-mutant strains with similar phenotypes are intercrossed, each of the four mutant progeny from nonparental ditype (NPD) asci must necessarily be a double mutant with mutant alleles at both loci.

Need may arise to construct double-mutant strains combining two mutant genes that have similar phenotypes, for example *white collar-1* and *white collar-2*, or *conidial separation-1* and *conidial separation-2*. For genes that are not closely linked, this can be readily accomplished by intercrossing the two single mutants and obtaining nonparental ditype (NPD) asci in which four of the ascospores are wild type recombinants and four are double mutants. For two genes that are closely enough linked so that NPDs are rare, a single crossover will produce a tetratype (T) ascus consisting of six mutant and two wild-type ascospores. If the two double-mutant recombinants in a tetratype ascus cannot be distinguished phenotypically from the four single-mutant parental types, progeny-tests or complementation-tests may be required.

The reverse situation may arise, where it is desired to recover a single-mutant component from a double mutant strain. This might occur when a suppressor or other modifier of a mutant gene has been obtained in combination with the suppressed mutation and it is desired to extract the suppressor and and determine its phenotype. Here, NPD asci from *double mutant* × *wild type* would be recognized by the presence of four nonsuppressed mutant ascospores. For example, a newly isolated suppressor of the *trp-2* mutation, *su(trp-2)*, could be separated from the original mutation by crossing it by wild type and using NPDs. A NPD ascus would contain four products that require tryptophan, while the remaining four products carried the suppressor mutation in combination with $trp\text{-}2^{+}$. A parental ditype (PD) ascus would contain four products that were genotypically $trp\text{-}2^{+}$*; su(trp-2)*$^{+}$ and four that were *trp-2; su(trp-2)*. None of the eight products would require tryptophan..

Procedure

Intercross the mutant strains to be tested on synthetic cross medium in a petri dish. (Use a heterokaryon with *helper-1* if the tested strains are female-sterile or if it is desired to avoid supplementing the cross

medium.) Collect unordered asci as described in "*How to obtain asci as unordered groups of ascospores ejected from the perithecium*". Isolate ascospores from each ascus to appropriate medium and score progeny as mutant or nonmutant.

Tetratype (T) asci are produced when single exchanges (or odd-numbered multiples) occur in the interval between two linked genes or in the intervals separating two unlinked genes from their centromeres. Consequently, the number of asci required to obtain an NPD will depend on the location of mutant loci relative to each other (for linked genes) or to the centromeres (for unlinked genes). When the map location of one or both genes is not known, a frugal plan would be to begin by isolating ascospores from seven asci, germinating them on complete or permissive medium, and scoring the germlings one ascus at a time to determine whether any of the asci are NPDs. The rationale for this is as follows: If the two genes are unlinked and both are at centromeres, there will be no tetratypes and half of the asci will be NDPs. This most favorable condition will rarely be met, and more than seven asci may be required. Obtaining an NPD will be most difficult when one or both loci are far out in the chromosome arm. Two-thirds of the asci will then be T's because of crossing over, and only one-sixth (half of the remainder) will be NPDs.

References

Davis, R. H. 1962. Consequences of a suppressor gene effective with pyrimidine and proline mutants of Neurospora. Genetics 47: 351-360.

Gavric, O., and A. J. Griffiths. 2003. Interaction of mutations affecting tip growth and branching in Neurospora. Fungal Genet. Biol. 40: 261-270.

Inoue, H. 1999. DNA repair and spedific-locus mutagenesis in *Neurospora crassa*. Mutat. Res. 437: 121-133.

Jarvik, J., and D. Botstein. 1973. A genetic method for determining the order of events in a biological pathway. Proc. Nat. Acad. Sci. USA 70:2046-2050.

Kafer, E. 1983. Epistatic grouping of repair-deficient mutants in Neurospora: comparative analysis of two *uvs-3* alleles *uvs-6* and their *mus* double mutant strains. Genetics 105: 19-33.

Moir, D., and D. Botstein. 19982. Determination of the order of gene function in the yeast nuclear division pathway using *cs* and *ts* mutants. Genetics 100: 565-577.

Seale, T. W. 1976. Supersuppressor action spectrum in Neurospora. Molec. Gen. Genet. 148: 105-108.

Srb, A. M. 1946. Ornithine-arginine metabolism in Neurospora and its genetic control. Ph. D. Thesis,. Stanford University. 46 p.

Srb, A. M., and N. H. Horowitz. 1944. The ornithine cycle in Neurospora and its genetic control. J. Biol. Chem. 154: 129-139.

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