

How to obtain uncontaminated progeny from crosses involving *per-1*.

David D. Perkins

Background

Ascospores carrying a type-1 mutant allele of the *per-1* (*perithecial -1*) gene are unpigmented, germinate spontaneously without heat shock or chemical activation, and they are killed by exposure to 60°C, the temperature normally used to activate wild type ascospores. Obtaining progeny from crosses involving *per-1* makes one appreciate the fact that the standard 60° heat shock kills all vegetative cells - - macroconidia, microconidia, and hyphal fragments. When *per-1* ascospores are isolated, the germlings they produce are liable to be contaminated with vegetative cells of parental origin.

Macroconidia can be eliminated as potential contaminants by using as female parent a strain that does not produce airborne conidia, and isolating unpigmented ascospores that have been shot to an agar surface. The aconidiate strain *fluffy* can be used, but it is not completely satisfactory because fully pigmented *fl: per⁺* ascospores show an appreciable rate of spontaneous ascospore germination. (Also, *fluffy* strains produce abundant microconidia, which are potential contaminants even though they do not become airborne.) If ascospores from a cross that is heterozygous for both *fl* and *per-1* are left without heat shock to form colonies on the agar surface of a plate, some of the colonies will have originated from *fl; per-1⁺* ascospores.

In contrast, the *conidial separation* mutants *csp-1* and *csp-2*, which do not release conidia into the air, do not exhibit spontaneous ascospore germination.. They are therefore preferred to *fluffy* as protoperithecial parents in crosses that involve *per-1*

Procedure

Crosses are best made in petri dishes using as the protoperithecial parent one of the strains that do not produce airborne conidia. Ascospores are collected by inverting the plate over an agar surface rather than taking them from the wall of the tube, which may have been overgrown with mycelia. The following strains are suitable. All are in OR background and the *per-1* allele is type I. See Perkins *et al.* (2001) for a description of these mutants.

<i>csp-1 A</i>	FGSC 2554
<i>csp-2 A</i>	FGSC 4085
<i>csp-2 a</i>	FGSC 4086
<i>per-1; csp-2 A</i>	FGSC 6662
<i>per-1; csp-2 a</i>	FGSC 6663
<i>fl A</i>	FGSC 4317
<i>fl a</i>	FGSC 4347
<i>fl; per-1 A</i>	FGSC 3311
<i>fl; per-1 a</i>	FGSC 3312

References

Howe, H. B., Jr., and T. E. Johnson. 1976. Phenotypic diversity among alleles at the *per-1* locus of *Neurospora crassa*. *Genetics* 82: 595-603.

Perkins, D. D., B. C. Turner, V. C. Pollard, and A. Fairfield. 1989. *Neurospora* strains incorporating *fluffy*, and their use as testers. *Fungal Genet. Newslett.* 36: 64-67.

Perkins, D. D., A. Radford, and M. S. Sachs. 2001. *The Neurospora Compendium: Chromosomal Loci*. Academic Press, San Diego.

Selitrennikoff, C. P. 1974. Use of conidial separation-defective strains. *Neurospora Newslett.* 21: 22.

DDP