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Neurospora strains incorporating fluffy, and their use as testers.

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Abstract
We have used fluffy (fl) strains extensively as female parents in mating-type tests and for a variety of other applications where high fertility and absence of conidia are advantageous.

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We have used fluffy (fl) strains extensively as female parents in mating-type tests and for a variety oi other applications where high fertility and absence of conidia are advantageous. Most of the procedures have been published, but the descriptions are scattered. We are therefore summarizing them here, together with a table of the strains currently used as testers for the various procedures.

The aconidiate fluffy testers have the advantage that tests can be carried out in small (10 x 75 mm) culture tubes. Conidiation precludes the use of wild-type strains as testers in the small tubes: if necessary they can be used in larger tubes (12 x 100 mm), where conidia are less likely to obstruct observations or to prevent ejected ascospores from reaching the wall of the tube.

Temperature for crosses is 25°C both before and after fertilization and the crossing medium is that of Westergaard and Mitchell as modified in Davis and de Serres (1970 Method Enzymol. 17A:79-143, P. 86, or see Neurospore News. 4:21-25, 1963). Stocks of the testers are preserved in silica gel. Working stocks are grown up on slants in 20 x 150 mm tubes, then stored at 5°C until use.

1. Determining mating type

a. Plates Inoculate tester to middle of plate containing crossing medium and incubate 4 days. (Length of incubation for maximum fertility depends on the depth of agar. Protoperithecia form more quickly on shallow plates.) Plates with lawns ready to fertilize may be stored at 5° for several weeks before use. Conidial suspensions from strains being tested are spotted on the fl lawn at defined positions. A loop of sterile water is used to pick up conidia from a slant of the culture to be tested, making sure that loose conidia do not adhere to the loop. At least 30 spots are easily accommodated per plate. If mating types differ, perithecia become visible in two or three days.

b. Slants. Inoculate slants of crossing medium (1 ml in 10 x 75 mm tubes) dropwise with a Pasteur pipet, using a suspension of fresh fl mycelia grown on either solid or liquid medium. The mycelia are homogenized in water by grinding with a sterile rod, pipet or tissue homogenizer. Incubate 4 days. If not used immediately, the tubes can be stored at 5° for at least 3 weeks. Fertilize using a short, stiff, dry platinum-iridium or nichrome needle hammered to form a flattened blade. The fertilizing conidia or mycelia should be distributed across the surface of the fluffy tester by rubbing with the blade. Mycelia may be present on the glass opposite the agar where they would interfere with observing perithecia and shot spores. If so, the tube wall should be cleared with a swipe of the blade at the time of fertilization. Perithecia appear in 2 or 3 days.

2. Examination of ejected ascospores for presence of aborted or abnormal ascospores. (Useful for mutants affecting ascospore color and shape, Spore killers, chromosome rearrangements [Perkins and Barry 1977 Adv. Genet. 19:133-285]. The background noise of white spores from other causes can be practically eliminated by choosing the right fluffy testers. See accompanying note and Table 1.) Prepare and fertilize small test slants as in lb. Spores are ejected to glass opposite the perithecia beginning at 10 days. Scan using 60-70x magnification and strong diffuse light from below. (We use Bausch and Lomb Stereo Zoom 7 with frosted substage mirror).

3. Scoring crosses as barren or fertile. (Useful for studying duplications, duplication-generating rearrangements and genes affecting fertility.) Crosses having impaired perithecial development so that spores are absent (or infrequent) are termed barren (Raju and Perkins 1978 Can. J. Genet. Cytol. 20:41-59). Proceed as in 2. If few or no ascospores are ejected at l0-12 days, examine perithecia for beaks. Perithecia devoid of mature asci usually remain round and fail to form beaks. Many mutagen-sensitive mutants are barren when homozygous (e.g. uvs-6 x uvs-6). Crosses in which one or both parents contain duplications are typically barren. With some duplications, barrenness is incomplete and a few ascospores are formed. With some, fertility is eventually regained in individual perithecia as duplicated segments are deleted. The most reliable incubation time for scoring barren progeny of duplication-generating rearrangements varies, depending on the rearrangement being studied (see Perkins and Barry 1977 Adv. Genet. 19:133-285). Perithecia are barren and rudimentary in most but not all interspecies combinations; the exceptions involve N. intermedia (see below).

4. Determination of species. Proceed as in 2. For timely identification of a small number of cultures, cross to fl A and fl a testers of N. crassa, N. intermedia and N. sitophila. A transfer to sterile synthetic crossing medium can be made at the same time to check for N. tetrasperma. Most but not all wild-collected N. tetrasperma isolates are self-fertile A+ a heterokaryons which will make perithecia with four-spored asci. To process a large number of unknown strains, or in a laboratory where only N. crassa is available, it is convenient to cross first to N. crassa. If ascospores are abundant and predominantly black, the species is N. crassa. If ascospores are produced but are sparse and predominantly pale and white, the unknowns should be crossed to N. intermedia fl of the appropriate mating type. If the cross to N. crassa produces small perithecia without ascospores, the unknowns should be crossed to N. sitophila fl (and to N. discreta and N. tetrasperma if necessary). fluffy testers are not needed for N. tetrasperma or for N. discreta because the wild-types used as species-testers produce few conidia at 25°C. N. tetrasperma wild types 85A(FGSC 1270) and 85a (FGSC 1271) give good results. E A (FGSC 5897) or E a (FGSC 5901) may be preferred as alternatives where it is advantageous to obtain small, homokaryotic ascospores. N. discreta Kirbyville wild types (FGSC 3228 and 4378) are satisfactory as testers on SC.

5. Scoring patterns of aborted ascospores in ejected asci. (Used for diagnosing chromosome rearrangements, Spore killers, and mutations visible in ascospores.) The frequencies of asci with different numbers of aborted ascospores are diagnostic of different kinds of chromosome rearrangements (Perkins 1974 Genktics 77:459-489; Perkins and Barry 1977 Adv. Genet. 19:133-285). These are best observed as groups of eight spores gathered on an agar collecting surface (Perkins 1966 Neurospora Newsl. 9:ll). Ascospore color mutants and Spore killers can be distinguished from rearrangements in this manner because the former produce asci of only one type, having four defective ascospores.

Use of fluffy as a protoperithecial parent avoids conidial scatter and the trapping of shot asci by overlying conidia. fluffy lawns are prepared in petri dishes as in la, taking care that the agar be deep enough to avoid desiccation during 2 weeks incubation. A central area 5 or 6 cm in diameter is fertilized confluently by either rubbing the inoculum onto the lawn with a blade or by pipetting a suspension. Plates are inverted and incubated in the dark for 10 days, then brought into the light for collecting asci. Shooting continues for several days. Ascospore octets are collected on a 4% agar-water slab placed on microscope slides which are stacked so that the collecting surface is within 1 mm of the ostioles, under the inverted cross plate. The exposure period may vary from 15 seconds to several minutes, depending on the rate of shooting. (See Perkins 1966 Neurospora Newsl. 9:11 for details of method.)

6. Scoring Spore Killer. The use of fl testers greatly facilitates scoring crosses involving Spore killer. Scoring is based on the identification of defective spores, which are often tiny and could be confused with conidia, particularly when large numbers of spores have been shot confluently. In crosses of SK^S x SK^K each ascus contains four normal-sized black spores and four white defective spores. Crosses that involve all other combinations of SK^K, SK^S and r(Sk) produce asci with all black spores. When SK^S and SK^K are segregating in a cross, the standard fl strains, which are SK^S, are used as testers. They are also used when SK^K and r(Sk) are segregating. When SK^S and r(Sk) are segregating in a cross, the appropriate SK^K fl testers are used.

7. Isolation of per ascospores. (Necessary when progeny containing per are to be recovered.) Type I alleles of the perithecial color mutant per are expressed autonomously in colorless per ascospores, which germinate spontaneously and are killed by 60°C heat shock as are all vegetative cells. To obtain uncontaminated per progeny it is therefore necessary to separate unheatshocked per ascospores from all conidia before isolating them to the medium on which they germinate and grow. If the female parent is fluffy, conidia are not produced and pure cultures can conveniently be obtained from ejected per ascospores. Use of fluffy for this purpose has one drawback: ascospores containing fl frequently germinate spontaneously without heat shock, even though they are per+. Care must therefore be taken to distinguish whether spontaneous germinants originated from unpigmented (per) or from pigmented (per+;fl) ascospores. The distinction can usually be made if ascospores are spread on 4% agar in a petri dish and picked to tubes after germination. A gene preventing conidial separation (csp-1 or csp-2) can be used instead of fluffy to prevent conidial scatter when working with per. The csp;per+ ascospores do not germinate spontaneously.

Table 1: *fluffy* testers in current use. Strains are *Sk*-sensitive unless indicated otherwise.

Genotype*	FGSC no.	Allele	Background	Applications
N. crassa				
fl(OR) A	4317	P	b7 x OR	Testing mating type, species, Spore killer. Detecting and scoring rearrangements in other than OR background.
fl(OR) a	4347	P	b7 x OR	
fl(RL) A	6682	P	b6 x RL	Testing mt, species, *Sk*. Detecting and scoring rearrangements in other than RL background.
fl(RL) a	6683	P	b4 x RL	
fl;Sk-2^K A	3297	P;K-Borneo	b9 x OR	Identifying strains resistant to killing.
fl;Sk-2^K a	6137	P;K-Borneo	b10 x OR	
fl;Sk-3^K A	3579	P;K-Rouna	bl0 x OR	Identifying strains resistant to killing.
fl;Sk-3^K a	3580	P;K-Borneo	bl0 x OR	
fl;per-1 A	3311	P;PBJl	f1 x OR	Use as female to avoid conidial contamination when spontaneously germinating *per-* ascospores are isolated. Used to show maternal origin of perithecial walls in reciprocal cross x *per+*.
fl;per-1 a	3312	P;PBJl	f1 x OR	
N. intermedia				
fl A	5798	P	b7, *crassa* » Shp	Testing mt, species, *Sk* or rearrangements
fl a	5799	P	b7, *crassa* » Shp	
N. sitophila				
fl;Sk-1^K+				
fl;Sk-1^S A	4887	P	b_5, *crassa* → *sitophila* FGSC 1134, 3191	Testing mt, species, *Sk*. for efficient rearrangement tests use K x K or S x S
fl;Sk-1^S a	4888	P		

* Strains of *N. crassa* and *N. intermedia* are sensitive to killing by *Sk-2^K* and *Sk-3^K* unless otherwise indicated. (OR) and (RL) signify strains backcrossed to Oak Ridge and to Rockefeller-Lindegren wild types. See accompanying note by Perkins and Pollard.

\+ Satisfactory *fl;Sk-1^K* stocks are not available. It is recommended that *N. sitophila* wild type Sk1^K stocks P8085 A (FGSC 2216) and P8086 a (FGSC 2217) be used instead, on slants in 12 x 100 mm tubes.

Precautions. Vegetatively propagated *fluffy* stocks deteriorate occasionally -- subcultures acquire undesirable traits such as decreased fertility, delayed protoperitheciation, or production of large brown protoperithecia (false perithecia). For example, Veenhuizen and Kolmark (Fungal Genet. Newsl. 33:46-47, 1986) noted lowered fertility and showed that it was due to a modifier; fully fertile *fluffy* strains were recovered in progeny when the modified strain was crossed to wild type. On occasion we also have made crosses to rid *fluffy* strains of modifiers responsible for false perithecia and slow sexual development. It should ordinarily be unnecessary to do this if well-behaved *fluffy* stocks are available that have been preserved in suspended animation. Aconidial strains such as *fluffy* do not survive freezing at -20°C. FGSC finds, however, that they can be simply and effectively preserved at -70°C. We routinely rely on silica gels from which fresh *fluffy* working stocks are made once or twice a year. The working stocks are kept at 5°C while being used as a source of inocula for tests. - - - Department of Biological Sciences, Stanford University, Stanford, CA 94305