

How to determine mating type.

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Background

Determining the mating type of segregants or newly acquired strains is a prerequisite for proceeding with genetic analysis. In heterothallic *Neurospora* species, crossing takes place only between strains of opposite mating type, while heterokaryons are normally formed only between strains of the same mating type. Crosses are required for building stocks, determining linkage, studying recombination, detecting and diagnosing chromosome rearrangements, and identifying the species of newly acquired isolates. Crosses are also required for detecting and studying meiotic mutants, Spore killer elements, and other variants that are expressed during the sexual phase. Crosses have been essential for discovering and analyzing repeat induced point mutation (RIP) and meiotic silencing of unpaired DNA (MSUD).

The mating type of an unknown isolate can be determined by crossing it to a fertile strain of known mating type. The highly fertile aconidiate mutant *fluffy* (*fl*) is superior to wild type for determining mating type. When used as protoperithecial (female) parents, *fluffy* strains form perithecia quickly and abundantly, and the perithecia are more readily observed than in crosses by wild type, where they may be obscured by the abundant overlying macroconidia. See *How to use fluffy testers for determining mating type and for other applications*.

If *mat A* and *mat a* are both available when cultures of a new mutation are sent to the Fungal Genetics Stock Center, they should both be deposited. Having them both available may expedite setting up crosses. The redundancy also provides insurance against loss of the mutation.

Procedure

Tests are conveniently made using the tester as female parent and spotting conidia or mycelial fragments from unknowns onto lawns of the appropriate testers. Thirty or more strains may be tested on a single petri plate. Alternatively, slants may be used. For instructions and practical details of methodology, see *How to use fluffy testers for determining mating type and for other applications*. Confidence in the results of mating-type tests is greatly increased if each isolate is tested on both *mat A* and *mat a*, enabling false negatives and errors to be detected.

fluffy testers are available in *N. crassa*, *N. intermedia*, *N. sitophila*, and *N. tetrasperma*. (Table 1). Strains other than *fluffy* are effective as testers in *N. tetrasperma* and *N. discreta*, as described below.

When strains from nature are being tested, the first step in ascertaining species of an isolate is to test it using *N. crassa mat A* and *mat a fluffy* testers. Reactions are seen with one *N. crassa* mating type but not the other in all species combinations except for *N. discreta*. Manifestation of the reaction varies depending on the species, from rudimentary and barren (*N. tetrasperma*, *N. sitophila*) to full size and productive (*N. intermedia*). See *How to determine the species of a wild-collected isolate*.

***N. tetrasperma*:** Because crossing over is blocked in the mating type chromosomes of *N. tetrasperma* (Gallegos *et al.* 2000, Jacobson 2005), a mutation at any *mat*-linked locus can be used to tag the *mat* idiomorph and reveal the mating type of monokaryotic progeny from a heterozygous cross. By tagging the mating type of one parent in a cross with a visible marker, the mating type of segregants can be determined by inspection, without crossing to testers. The readily scored *al-2* allele 102 has been commonly used to tag *mat A*. Strains with *mat*-linked markers other than *al-2* are listed in the FGSC catalog under "*N. tetrasperma* markers".

In absence of a tag, test crosses will be required. Conidiation does not usually obscure perithecia in wild type *N. tetrasperma*, so it is not essential for testers to be aconidiate. However, a mutant *fluffy* allele has been introduced into *N. tetrasperma* from *N. crassa*, providing monokaryotic *mat A* and *mat a fluffy* strains that can be used as mating type testers if desired (Table 1).

Howe (1964) noted that the *mat A* and *mat a* components of *N. tetrasperma* strain 85 differed in *mat*-linked visible traits and that these could be used to identify the mating type of progeny in crosses with parents derived from strain 85. Howe (1961) had previously attempted to avoid the labor of mating-type tests in *N. crassa* by tagging mating type with the closely linked marker *un-3*. However, the labor of inserting an extraneous mutant marker in one parent, and scoring the marker outweighed any advantage it might confer. Thus, mating type in *N. crassa* and other heterothallic *Neurospora* species continues to be scored by crossing to testers rather than by using a *mat*-linked tag.

N. discreta: *fluffy* testers are not available and they are probably not needed. Tests can be carried out on lawns or 12 x 110 mm slants using the Kirbyville species-type strains, FGSC 3228 and 4378 (Table 1). See Jacobson *et al.* (2004). See: *How to determine the species of a wild-collected isolate*.

References

- Gallegos, A., D. J. Jacobson, N. B. Raju, M. P. Skupski, and D. O. Natvig. 2000. Suppressed recombination and a pairing anomaly on the mating-type chromosome of *Neurospora tetrasperma*. *Genetics* 154: 623-633.
- Howe, H. B., Jr. 1961. Determining mating type in *Neurospora* without crossing tests. *Nature* 190: 1036.
- Howe, H. B., Jr. 1964. Vegetative traits associated with mating type in *Neurospora tetrasperma*. *Mycologia* 56: 519- 525.
- Jacobson, D. J. 2005. Blocked redomination along the mating-type chromosomes of *Neurospora tetrasperma* involves both structural heterozygosity and autosomal genes. *Genetics* (In press.).
- Jacobson, D. J., A. J. Powell, J. R. Dettman, G. S. Saenz, M. M. Barton, M. D. Hiltz, W. H. Dvorachek, Jr., N. L. Glass, J. W., Taylor, and D. O. Natvig. 2004. *Neurospora* in temperate forests of western North America. *Mycologia* 96: 66-74.

Table 1. Testers for determining mating type.

Genotype ^a	FGSC No.	Allele or Strain No.
N. crassa ^b		
fl(OR) A	4317	P
fl(OR) a	4347	P
N. intermedia		
fl A	5798	P
fl a	5799	P
N. sitophila		
fl A	4887	P
fl a	4888	P
N. tetrasperma		
al-2 A	1256	102
85 a	1271	85
fl A	7084	P
fl a	7085	P
N. discreta		
Kirbyville A	3228	P846
Kirbyville a	4378	P8127

These strains are recommended if the purpose of the test is simply to determine or confirm mating type. If uses other than mating-type determination are contemplated, Table 1 of "*How to use fluffy testers for determining mating type and for other applications*" lists alternative *mat A* and *mat a* testers that might be preferred to those given here.