Wild type *Neurospora crassa* strains preferred for use as standards

David D. Perkins, Department of Biological Sciences, Stanford University, Stanford CA 94305-5020

Fungal Genet. Newsl. 51:7-8

The highly inbred *Neurospora crassa* strains 74-OR23-1VA (FGSC 2489) and 74-ORS-6a (FGSC 4200) are recommended for use as standard wild types.

Strains of Oak Ridge genetic background that are largely coisogenic were obtained by backcrossing and vegetative reisolation (Mylyk *et al.*, 1974; Kafer, 1982). These strains, 74-OR23-1VA (FGSC 2489) and 74-ORS-6a (FGSC 4200), have been preferred for use as standards. Now, using them seems more desirable than ever because 74-OR23-1VA was the source of DNA used to sequence the Neurospora genome (Galagan *et al.*, 2003).

Although the improved strains have been available for two decades, some laboratories continue to employ their predecessors, which are no longer preferred but which are still listed by FGSC for archival purposes. 74 OR8-1a is one of the ancestral strains that should be avoided because it differs from the ORA strains in several respects (Mylyk *et al.*, 1974).

The current Oak Ridge standards are descended from ST74A, STA4, and ST73a, strains that Patricia St. Lawrence selected as standards on the basis of their favorable qualities for meiotic chromosome cytology and genetics (St. Lawrence, 1953). For a pedigree showing the lineage of the now-preferred standards and their relation to the strains they replace, see Newmeyer *et al.* (1987). 74 ORS-6a was the product of six recurrent backcrosses to 74-OR23-1VA (Kafer, 1982), beginning with ORSa. The ORSa strain was itself the product of seven recurrent backcrosses to 74-OR23-1A, beginning with 74 OR8-1a (Mylyk *et al.*, 1974). The only function of the prefix "74-" is to show that all of these OR strains are alike in having been derived from backcrosses to ST74A or its *mat A* descendents. It has therefore been deemed unnecessary and has often been omitted from numbers designating the OR wild-type strains.

The heterokaryon incompatibility genotype of the two recommended standards is *het-C, het-d; het-e.* By definition they also carry the OR alleles of *het-6* (*het-6*^{OR}) and of all het loci discovered subsequently (Mylyk, 1975). Whether a strain is heterokaryon-compatible with Oak Ridge strains can readily be tested by determining whether it complements one or another of the OR-compatible helper strains. *helper-1* (*mat a*^{m1} *ad-3B cyh-1*, FGSC 4564) is useful for testing on minimal medium if the strain to be tested contains a selectable auxotrophic marker (Perkins, 1984). Alternatively, the FUDR-sensitive tester *helper-2* (*mat*Δ *tk*$^{+}$(FUDR^{S}) *cyh-1; inl; Bml pan-2*, FGSC 8745) can also be used even if the strain to be tested contains no selectable auxotrophic marker. Testing is then done on minimal medium + Benomyl (Metzenberg and Sachs, 2002).

If aconidiate strains in Oak Ridge background are desired, the OR-compatible fluffy testers *fl* (OR) A (FGSC 4317) and *fl* (OR) a (FGSC 4347) are recommended (Perkins *et al.*, 1989).

Precautions:

(1) Cultures of the OR wild type strains are reported to become heterokaryotic by acquiring spontaneous mutations that resemble the *soft* (*so*) mutation (Kafer, 1982). Occasional replacement with an authentic stock from FGSC, or purification of stocks by vegetative reisolation, may therefore be desirable.

(2) Inbreeding has apparently fixed genetic factors that result in nonselective abortion and disintegration of asci ("bubble asci") in crosses between OR strains (Raju *et al.*, 1987). Although as many as 70% of asci may degenerate, fecundity of OR A X OR a crosses is not reduced enough to impede genetic analysis. If desired, outcrosses of strains in OR background to strains such as RL can be used to reduce or eliminate ascus abortion. Strains *fl* (RL) A, (FGSC 6682), *fl* (RL) a (FGSC 6683) or RL3-8 A (FGSC 2218), RL21 a (FGSC 2219) are recommended for this purpose (Perkins and Pollard, 1989). All four RL strains carry the gene *scot*, which is cryptic at 25°C but results in abnormal, erratic growth above 34°C (Perkins and Björkman 1978). For this reason, and because the RL strains differ from the OR standards in their het-genotype, progeny from the RL testers should probably not be saved.

References

J, E. Galagan, S. E. Calvo, K. A. Borkovich, *et al.* 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. Nature 422:859-868.

Kafer, E. 1982. Backcrossed mutant strains which produce consistent map distances and negligible interference. Neurospora Newslett. 29: 41-44.

Metzenberg, R L., and M. Sachs. 2002. Neurospora heterokaryons involving a thymidine kinase-positive "helper": Use in storing poorly viable strains or crossing strains of limited fertility. Fungal Genet. Newslett. 49: 19.

Metzenberg, R. L., J. N. Stevens, E. U. Selker, and E. Morzycka-Wroblewska. 1984. A method for finding the genetic map position of cloned DNA fragments. Neurospora Newslett. 31: 35-39.

Mylyk, O. M. 1975. Heterokaryon incompatibility genes in *Neurospora crassa* detected using duplication-producing chromosome rearrangements. Genetics 80: 107-124.

Mylyk, O. M., E. G. Barry, and D. R. Galeazzi. 1974. New isogenic wild types in *N. crassa*. Neurospora Newslett. 21: 24.

Newmeyer, D., D. D. Perkins, and E. G. Barry. 1987. An annotated pedigree of *Neurospora crassa* laboratory wild types. showing the probable origin of the nucleolus satellite and showing that certain stocks are not authentic. Fungal Genet. Newslett. 34: 46-51.

Perkins, D. D. 1984. Advantages of using the inactive-mating-type a^{ml} strain as a helper component in heterokaryons. Neurospora Newslett. 31: 41-42.

Perkins, D. D., and M. Björkman. 1978. A temperature-sensitive morphological mutant present in Beadle-Tatum and Rockefeller-Lindegren "wild-type" stocks and their derivatives. Neurospora Newslett. 25: 24-25.

Perkins, D. D., and V. C. Pollard. 1989. Alternate *fluffy* testers for detecting and diagnosing chromosome rearrangements in *Neurospora crassa.* Fungal Genet. Newslett. 36: 63-64.

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. , J. F. Leslie, and D. J. Jacobson. 1993. Strains for identifying and studying individual vegetative (heterokaryon) incompatibility loci in *Neurospora crassa*. Fungal Genet. Newslett. 40: 69-73.

Raju, N. B., D. D. Perkins, and D. Newmeyer. 1987. Genetically determined nonselective abortion of asci in *Neurospora crassa*. Can. J. Bot. 65: 1539-1549.

Slayman, C. W., and E. L. Tatum. 1964. Potassium transport in Neurospora. 1. Intracellular sodium and potassium concentrations, and cation requirements for growth. Biochim. Biophys. Acta 88: 578-592.

St. Lawrence, P. 1953. The association of particular linkage groups with their respective chromosomes in *Neurospora crassa*. Ph. D. Thesis. Columbia University. 152 p. University Microfilms. Ann Arbor, Mich. (L. C. Card No. Mic A54- 1 1) Dissertation Abstr. 14: 7-8 (1954).