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Abstract

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Alternate *fluffy* testers for detecting and diagnosing chromosome rearrangements in *Neurospora crassa*

We have long used *fluffy* strains as testers in detecting, scoring and analyzing chromosome rearrangements. The fluffy strains are advantageous because of high fertility and the absence conidia, which would impair observation of ejected ascospores. The proportions of ascus types having various numbers of aborted ascospores are diagnostic of particular kinds of rearrangements (Perkins 1974 Genetics 77:459-489). An excess of white ascospores among those ejected from the perithecia signals presence of a rearrangement in crosses of a tested strain to the normal-sequence *fluffy* tester.

The *fluffy* testers used as standards in most of our work have been essentially isogenic with Oak Ridge (OR) wild types. The one disadvantage of these testers is that they give 5-10% white, inviable ascospores in many crosses where no chromosome rearrangement is present. This level of background noise does not interfere with recognizing cleat-cut rearrangements such as reciprocal translocations, where 50% of ascospores from Translocation x Normal fail to pigment because they contain lethal deficiencies. The 5 to 10% background of white spores is sometimes bothersome with rearrangements such as insertional translocations, where only 25% of ascospores contain deficiencies. Identifying the presence of such a translocation in the test procedure requires distinguishing 75% from 90% black ascospores among those ejected to the wall of a cross tube. This is usually not difficult, but with some rearrangements the inviable deficiency ascospores darken somewhat, increasing the difficulty. The chief concern, however, is that the high background of white ascospores might obscure the presence of rearrangements such as short inversions, where fewer than 25% of ascospores are expected to be rendered colorless by presence of the heterozygous aberration.

We now know that the background frequency of white ascospores in crosses without rearrangements is related to inbreeding. Crosses between inbred strains produce more defective spores than do crosses between unrelated strains. The discovery came as an offshoot of a study of bubble asci (Raju, Perkins and Newmeyer 1987 Can. J. Bot. 65:1539-1549). In bubble asci, the entire ascus aborts and contains eight small degenerate vacuolated ascospores which are never ejected. Inbred crosses produce up to 70% bubble asci while outbred crosses produce less than 10%. Genetic analysis indicated that the frequency of bubble asci was the combined result of several weakly deleterious recessive genes. Crosses with a high percentage of bubble asci also produce an elevated number of inviable white ascospores in the ejected, nonbubble asci. Thus in inbred crosses, 5 to 10% of ejected ascospores are white, while outbred crosses produce as few as 1% white ascospores, or less (see Fig. 6 of Raju *et al.* 1987).

Since most of our rearrangements have arisen in OR strains or have been introduced into the OR background, it seemed advantageous to develop new fluffy testers with an unlike genetic background that would minimize the number of spontaneously produced white ascospores. The Rockefeller-Lindegren (RL) wild types developed by Garnjobst and Tatum were known to give nearly 100% black ascospores when crossed by Oak Ridge (although RL x RL produced 5 to 10% white spores among those ejected). Strains bearing the fl^P allele, originally in OR, were therefore backcrossed recurrently to RL wild types. After three or four generations the fl progeny produced 96% or 99% black ascospores when they were fertilized by OR wild types.

The new fluffy testers from these backcrosses are recommended for detecting, scoring, and analyzing rearrangements when the strain being tested is in the Oak Ridge background.

While the new testers are superior for use with rearrangements, their usefulness is limited in other respects. Like the RL wild types, the RL fluffy testers have the disadvantage of carrying the scot mutation, which results in abnormal growth and morphology at 34°C and above (Perkins and Bjorkman 1978 Neurospora Newsl. 25:24-25). scot (temperature sensitive spreading colonial) does not interfere with their usefulness crosses, which are carried out at 25°C. The RL fluffy testers probably also differ from the OR testers in heterokaryon incompatibility genotype, since RL is het-C het-D het-E and OR is het-C het-d het-e (Wilson and Garnjobst 1966 Genetics 53:621-631; Wilson, personal communication). Because of these and other potential differences in genetic background, we recommend that progeny of crosses parented by the new testers not be put in stock or used in further crosses unless preservation of the OR genetic background is of no concern.

We propose to distinguish the two sets of testers by inserting initials to specify wild-type background so that the old testers are designated fl (OR) A (FGSC 4317) and fl (OR) a (FGSC 4347), while the new testers are fl (RL) A (FGSC 6682) and fl (RL) a (FGSC 6683).

Both sets of fluffy testers are equally fertile and useful as species testers or as mating-type testers.

Practical aspects of using fluffy testers are outlined in an accompanying note. --

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