High throughput mating tests in *Neurospora crassa*

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Fungal Genetics Newsletter 53:20-22

Mating type tests in *Neurospora crassa* can be laborious and tedious. Since we were arraying mutants for distribution, we have developed a method to carry out mating type tests in 96-well plates. This technique is highly efficient and reproducible and gives results in three to four days.

Mating type tests in *Neurospora crassa* are an important way to characterize strains. Since most of the knock-out mutants developed as part of the functional genomics program (Colot *et al.*, 2006) have little obvious phenotype we have undertaken to test the mating type of all of the knock-strains that are sent to the FGSC. Our original mating type test protocol is similar to that described by Smith (1962) and involves growing a lawn of the fluffy tester strains (4317 or 4347) on cornmeal agar (Difco) in 15 cm petri plates. This is also described in the online Neurospora protocol, "How to use fluffy testers for determining mating type and for other applications" (http://www.fgsc.net/Neurospora/NeurosporaProtocolGuide.htm). The strains to be tested are grown on Vogels minimal (Vogel, 1956) or appropriately supplemented medium (McCluskey, 2003) and small amounts of conidia are transferred to a spot on a grid. Using this technique, thirty to forty strains can be tested on two plates. While robust, this technique is labor intensive and because the plate is opened for each inoculation there is the possibility that occasional stray conidia could confound the results.

To avoid introducing a rate limiting step into the processing of strains at the FGSC, we have implemented a mass-inoculation protocol to carry out mating tests in 96-well plates. Mating plates were prepared by aliquoting approximately 75ul of sterilized cornmeal agar (Difco) into each well of a 96-well plate. This medium was chosen because it supports rapid but thin growth of fluffy mycelia and is readily available. To carry out the mating test, we prepared a suspension of mycelial fragments and micro-conidia from seven to ten day old slant cultures of strains FGSC 4317 (*fl; mat a*) and 4247 (*fl; mat A*) by flooding a 5-10 day old slant with water and then macerating the mycelia using a sterile weighing spatula. Ten micro-liters of this suspension (containing 5 - 20 mycelial fragments as observed with a compound microscope) was transferred into each well of a prepared cornmeal agar plate using a 12-channel pipettor. The plates were incubated for 16 to 36 hrs at room temperature. Conidial suspensions of knock-out mutants in 3.5% sterile non-fat dry milk, 25% glycerol were prepared in a 96-well plate. These were either frozen at -80 or

Figure 1. Mating between strains on knock-out plate 9 and FGSC 4347

tested directly before freezing. Frozen samples were thawed at room temperature. Matings were initiated by transferring cells from the arrayed mutant plate to the mating tester plate using a 96-pin replicator (Boekel). The plates were incubated at 25C and were checked daily thereafter. Mating reactions were scored after two to three days and the plates were imaged by scanning on a flat-bed scanner after three days (Figure 1). The crosses went to completion and ascospores were present after several weeks at room temperature.

The mating reaction was clearly developed after two days and perethecia had started to darken. The appearance of mating was not confused by the presence of protoperithecia as shown in figure 2. Well H7 shows abundant perithecia while well H8 shows protoperithecia.

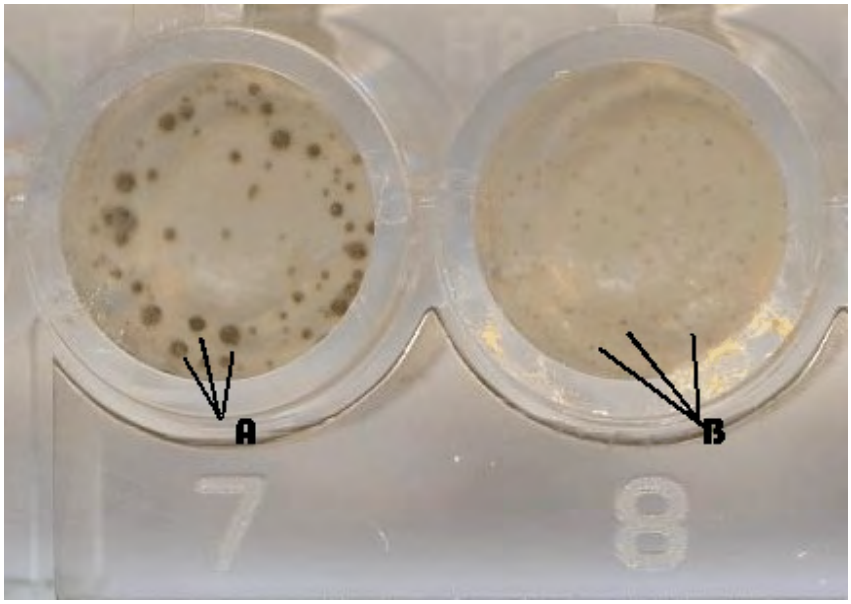

Figure 2. Plate mating between FGSC 4347 and strains FGSC 11983 (*mat A*, well H7) or 11984 (*mat a*, well H8). "A" indicates perithecia in well H7 while "B" indicates protoperithecia in well H8.

Seven hundred and eighty two strains on nine plates were tested. Some plates are not full because strains are not always submitted in numerical order. Of the 782 mutants, there were a total of 108 false negative reactions (approximately 14%) and sixteen false positive reactions (2%). The number of false negatives was inflated by the mating reaction between strain 4347 and mutants on plate 7 . In this case there were 21 false negatives, all of which successfully mated on standard testing plates (Table 1). This one bad plate could have been due to poor inoculation, or other handling issues such as the heating of the replicator by flaming. There were eleven strains that tested as the wrong mating type. The depositor of these strains indicated that we should change their mating types to reflect the testing results at the FGSC. One hundred eleven of the 124 were confirmed to be the appropriate mating type by mating on standard tester plates as described above. Using a 96-channel pipettor for both the fluffy and mutant inoculations could streamline this protocol but this equipment is not available at the FGSC. We did not use the 12 channel pipettor to introduce the mutant inoculum both because of the expense of the tips and because of the added time and manipulations.

Table 1. False mating reactions out of 782 strains tested.

FGSC # 4317		FGSC # 4347	
Preliminary False Negative	Preliminary False Positive	Preliminary False Negative	Preliminary False Positive
31	6	78	10
Mating type re-confirmed on plate test		Mating type re-confirmed on plate test	
25	4	71	7
Final False Negative	Final False Positive	Final False Negative	Final False Positive
6	2	7	3

Acknowledgements. The FGSC is supported by grant 0235887 from the US National Science Foundation and by National Institutes of Health Grant P01 GM068087.

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