

Perkins, D. D. Linkage testers having markers near the centromere.

Strains have been constructed that contain readily scorable mutant markers near the centromere in six linkage groups. Mating type marks the remaining group. Although the markers are not ideal for efficiency of scoring in all combinations, experience has shown these multicentromere test-

err to be quite useful, especially for situations where *olcoy* is not effective, as with temperature-sensitive mutants (because *cot-1* is a marker in *olcoy*), with mutants where *olcoy* fails to detect linkage (because *olcoy* markers are for distal in two linkage groups and VII is unmarked), and with chromosome rearrangements (because three translocations are already present in *olcoy*).

The following genes are present as markers:

Markers:	<u>A/a</u>	<u>bal</u>	<u>acr-2</u>	<u>pdx</u>	<u>at</u>	<u>ylo-1</u>	<u>wc</u>
	mating type	balloon	acriflavine	pyridoxine	attenuated	yellow	white collar
Isolation No. :		B56	KH5	37803	M111	Y30539y	PB29
Linkage group:	I	II	III	IV	V	VI	VII

Four stocks have been deposited with the Fungal Genetics Stock Center:

Multicent (all markers) A and a FGSC# 2014 and 2015, respectively
Multicent (without *at*) A and a FGSC# 1085 and 1086, respectively.

Our normal procedure with multicent is as follows: Use multicent as fertilizing parent. Suspend mycelial fragments in 1 ml water in a 10 x 75 mm tube, using a pipette to homogenize by grinding against the wall. Multicent can with some persistence be used as protoperithecial parent, but perithecia are slow to develop.

Isolate 100-150 ascospores to minimal + pyridoxine 10 days after spores start shooting. Germinate at 34°C.

Sort for bal, at and wc at 3 and 4 days. Set up scoring sheets and number tubes at 4 days. Because balloon grows as a restricted colony, it is easiest to work only among the bal⁺ half of the progeny, even though this requires that more spores be isolated originally.

at is readily scorable on minimal (with or without supplements) at 2 or 3 days (34°C), but conidiates more profusely on a complete medium, creating some scoring difficulties in older cultures. Growth is flat on the surface, with scattered specks of conidiation.

wc is clearest at temperatures above 25°C, and is scorable by the absence of carotenoids in mycelia, though not in conidia. Germinants are best kept at 34°C for 3 or 4 days under illumination till wc scoring is accomplished (usually in two readings 24 hours apart; ovoid reading just after cultures are brought from dark into light). Germinants are then moved to 25°C, where increased development of pigment in wc facilitates the scoring of ylo.

Unlike Carotenoid scoring improves with age, and is likely ylo-1 unreliable in young cultures. ylo-1 scoring at 3 or 4 days should be considered preliminary, and should be checked later.

acr-2 is scored clearly by transfer to min + 10 µg pyridoxine/ml + 50 µg acriflavine/ml.

pdx is most easily scored by transfer to min + 100 µg desoxypyridoxine·HCl/ml. It can be scored satisfactorily on minimal without the antagonist if sufficiently small inocula are used.

If linkage is not shown to markers in II - VII, mating type is scored on fl^{PA} and fl^P a testers, either by spotting onto 7-day old SC plates, SC plates, or by fertilizing 75 mm fl tube. (Tubes rather than plates are always used for chromosome rearrangements, so that isolates can be scored as Normal or Aberration sequence according to the presence of white deficiency ascospores among those shot to the wall of the tube.)

Effort is minimized by the stepwise scoring procedure. If on unmapped point mutant is scored early in the sequence, growth tests for pdx and acr-2 are required only if linkage to the visible markers is not apparent. Mating-type tests are then required only if no linkage is apparent to pdx or acr-2. With translocations, the normally independent multicent markers are examined for linkages to one another. ■ ■ ■ Department of Biological Sciences, Stanford University, Stanford, California 94305.

Letter to the Editor:

Over the past few years, I have been asked several times about the alleles of *crisp*, of osmotic and of *crisp*, osmotic that were used in the work by Trevithick and Metzberg (1966 Molecular sieving by *Neurospora* cell walls during secretion of invertase isozymes. J. Bacteriol. 92:1010.) *crisp* was allele B123, and osmotic was allele E11200. Unfortunately, we do not have a record of the allele numbers of the double mutant. It might have been B123, E11200, or it might have been B122, B135, since we once obtained this double mutant from the Fungal Genetics Stock Center; this detail is now lost in antiquity. Fortunately, the single mutants, from which most of the experimental information was obtained, can be identified with certainty. ■ ■ ■ R. L. Metzberg, Department of Physiologic.1 Chemistry, University of Wisconsin, Madison, Wisconsin 53706.