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

Strains have been constructed that contain readily scorable mutant marken 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 testerr to be quite useful, especially for situations where olcoy is not effective, as with temperature-sensitive mutants (because cot-l is a marker in olcoy), with mutants where olcoy foils to detect linkage (because olcoy markers ore for distal in two linkage groups and VII is unmarked), and with chromosome rearrangements (because three translocations are already present in alcoy).

The following genes ore present as markers:

Markers:	A/a mating type	bal balloon	acr-2 acriflavine	pdx pyridoxine	at attenuated	ylo-1 yellow	WC white collar
Isolation No. :		B56	KH5	37803	M111	Y30539y	PB29
Linkage group:	I	II	111	IV	V	VI	VII

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

Multicent (all markers) A and a FGSC# 2014 and 2015, respectively
Multicent (without&l) A ond 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 con with some persistence be used as protoperithecial parent, but perithecia ore slow to develop.

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

Sort for bol, at and wc at 3 ond 4 days. Set up scoring sheets and number tuber 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 thot 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 conidiotion.

wc is clearest at temperatures above 25°C, and is scorable by the absence of carotenoids in mycelia, though not in conidia. Germinants ore 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 ore brought from dark into light). Germinants ore then moved to 25°C, where increased development of pigment in wc facilitates the scoring of ylo.

Unlike Carotenoidscoring improves with age, and is likely ylo-1 unreliable in young cultures. o k or at first, then become yellow. 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 + IO µg pyridoxine/ml + 50 µg acriflavine/ml.

pdx is most easily scored by transfer to min + 100 µg desoxypyridoxine·HCl/ml. It con 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 tuber. (Tubes rather than plates are always used for chromosome rearrangements, so that isolates con 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 ore required only if linkage to the visible markers is not apparent. Mating-type tests ore then required only if no linkage is apparent to pdx or ocr-2. With translocations, the normally independent multicent markers ore 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 hove been asked several times about the alleles of crisp, of osmotic and of crisp, osmotic that were used in the work by Trevithick and Metzenberg (1966 Molecular sieving by Neurospora cell walls during secretion of invertase isozymes. J.Bacteriol. 92:1010.) crisp was allele B123, and osmotic was allele E 11200. Unfortunately, we do not have a record of the allele numbers of the double mutant. It might hove been B123, E11200, or it might hove 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, con be identified with certainty. - - - R. L. Metzenberg, Deportment of Physiologic.1 Chemistry, University of Wisconsin, Madison, Wisconsin 53706.