New multicent linkage testers for centromere-linked genes and rearrangements in Neurospora

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A tester described in 1972 contained readily-scored markers near the centromeres of all seven linkage groups (Perkins, Neurospora Newsl. 19:33). That tester, now called *multicent-1*, was somewhat more laborious to score than *alcoy* (Perkins et al. 1969 Genetica 40:247-278) or its successor *alcoy;csp-2* (Perkins and Björkman 1979 Neurospora Newsl. 26:9-10). *multicent-1* had the advantage over *alcoy* of requiring no follow-up cross to distinguish alternatives, and it was somewhat more effective in detecting linkage of left-arm markers. Because *alcoy* itself contains three translocations, *multicent-1* was more likely to identify the chromosomes involved in new centromere-linked translocations (Perkins and Barry 1977 Adv. Genet. 19:133-285).

One disadvantage with *multicent-1* was the marker *balloon*, whose restricted colonial growth meant that most of the other markers could be scored readily only in the *bal*+ half of the progeny. *bal* was also inconvenient for fertilization and stock preservation. We have therefore developed three new multicent testers. These are designated *multicent-3* to *-5* (Table I). Independently, Metzenberg et al. (1984 Neurospora Newsl. 31:35-39) devised a strain designated *multicent-2*, with six centromeres marked. This has been used for mapping cloned DNA fragments.

Table I. Constitution of multicent testers 1 through 5

Strain Designation	I	II	III	IV	V	VI	VII
multicent-1	*A* or *a*	*bal*	*acr-2*	*pdx*	*at*	*ylo-1*	*wc-1*
multicent-2	*un-2* *a*	*arg-5*	*thi-4*	*pyr-1*	*lys-1* *inl*		*ars-1* *nic-3*
multicent-3	*In(IL;IR)OY323* *A* or *a*	*arg-5*	*acr-2*	*pdx*	*at*	*ylo-1*	*wc-1*
multicent-4	A or *a*	*arg-5*	*acr-2*	*psi*	*at*	*ylo-1*	*wc-1*
multicent-5	*In(IL;IR)OY323* *A* or *a*	*arg-5*	*acr-2*	*psi*	*at*	*ylo-1*	*wc-1*

FGSC Numbers			helper*	
	A	a	A	a
multicent-1	2014	2015		
multicent-2		4488		
multicent-3	6824	6825	6826	6827
multicent-4	6828	6829	6830	6831
multicent-5	6832	6833	6834	6835

See FGSC stock list for allele numbers of markers.

* In heterokaryon with inactive mating helper strain *am1 ad-3B cyh-1*, FGSC 4564 (Perkins 1984 Neurospora Newsl. 31:41-42. The phenotypically wild type heterokaryon can be used as femal parent on minimal medium, but the *am1 ad-3B cyh-1* component does not participate in the cross

The *multicent-3* tester incorporates two changes. *arg-5* is substituted for *bal* in linkage group II, and a long inversion, OY323, has been inserted as a crossover-suppressor in linkage group I. Progeny are isolated either to complete medium or to minimal supplemented with arginine and pyridoxine. As a result of the heterozygous inversion, mating type shows little or no recombination with markers throughout two-thirds of I, which is the longest linkage group.

In *multicent-4* also, *arg-5* is substituted for *bal*. In addition, *psi* replaces *pdx* as a marker for IV. *psi* (protein-synthesis-inhibited) is a readily-scored temperature-sensitive conditional mutant that grows normally on minimal medium at 25°C but does not grow at 34°. Ascospores must be germinated at 25° because *psi* progeny do not survive at the restrictive temperature. Replacing *pdx* with *psi* means that only two media are required. *arg-5* is scored by transferring progeny to minimal medium at 25°, *psi* by transfer to arginine-supplemented minimal at 34°.

multicent-5 differs from *multicent-4* only in having the OY323 inversion present in linkage group I. *multicent-5* is preferred for mapping mutants that are scorable by vegetative phenotype. As with the other testers, unmapped translocations can be recognized by linkage between markers that are normally independent. If it should be desired to determine marker-breakpoint linkage by scoring for aborted ascospores in progeny tests, it is possible to score the new rearrangement only in the noninversion half of the progeny; these are recognized as noninversion because they are of the same mating type as the noninversion parent.

Scoring for the other markers is as follows: Tests for mating type are most readily accomplished on fluffy lawns in petri dishes (Perkins et al. 1989 Fungal Genet. Newsl. 36:64-66). *at* (attenuated morphology) is readily scorable on minimal (with or without supplements) at two or three days (34°C). Growth is flat on the surface, with scattered specks of conidiation. *wc-1* (white collar) is expressed most clearly at 25°C or higher. Carotenoids are absent in mycelia, though not in conidia. Germinants are incubated until conidia become orange, preferably under illumination. Scoring of *ylo-1* (yellow) improves with age, and is likely to be unreliable with young cultures, especially in combination with *wc*. Carotenoids in *ylo-1* look orange at first, then become yellow. *ylo-1* scoring at three or four days should be considered preliminary, and should be checked later. *acr-2* is scored clearly by resistance to 50 µg acriflavine/ml on solid medium.

With any of the testers, progeny are scored for markers sequentially beginning with the visible markers *at, wc-1,* and *ylo-1*. If linkage is apparent, the remaining markers need not be tested. If the unmapped mutant is unlinked to a visible marker, the markers that require transfer are then tested serially until linkage is seen. With translocations, the normally independent multicent markers are examined for linkages to one another.

The new multicent testers are heterokaryon-compatible with strains of OR background (*het-c het-d; het-e*). All three are available as heterokaryons in combination with the inactive-mating-type "helper" strain *am1 ad-3B cyh-1* (Table I). The heterokaryons are phenotypically wild-type

and highly fertile. Although the homokaryotic testers can be used satisfactorily for crossing, using a heterokaryon as female parent saves labor by making it unnecessary to supplement the crossing medium. Fertility may also be improved when the heterokaryon is used.