How to use multiply marked *multicent* strains for mapping genes and translocation breakpoints.

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Background

Normal-sequence linkage testers have been constructed that contain readily scored markers near the centromeres of all seven linkage groups (Table 1; Perkins 1972, 1990). While these *multicent* testers can be used for assigning unmapped genes to linkage group, their main value in our hands has been to identify the chromosomes involved in new centromere-linked translocations, which are recognized because they result in linkage between markers that are normally independent. The first choice for mapping new genes is usually *alcoy; csp-2* (Perkins and Björkman 1979), which has fewer and more easily scored markers than those in the *multicent* testers. However, because three unlinked translocations are already present in *alcoy; csp-2*, crosses may fail to reveal the linkages of a new, unmapped translocation that involves one of the same chromosome pairs that is already translocated in the tester.

One disadvantage with the original *multicent-1* tester was presence of a colonial marker *bal* (*balloon*) which interfered with scoring other markers except in bal^+ progeny. Modified testers without balloon were therefore constructed and designated *multicent-3* to *-5* (Table 1). (Metzenberg *et al*., 1984, had independently created *multicent-2* for use in RFLP mapping.)

Procedure

Heterokaryons of *multicent* strains with the inactive-mating-type *helper-1* strain a^{m1} *ad-3B cyh-1* (FGSC 4564; Perkins 1984) are phenotypically wild type and are fertile as female parents on unsupplemented crossing medium. (The *helper-1* component does not participate in the cross. See *How to use alcoy for linkage group assignment*.)

The *multicent-3* tester incorporates two changes: *arg-5* is substituted for *bal* in linkage group II, and a long inversion, *OY323*, is 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 LG I, the longest linkage group.

The *multicent-4* tester also has *arg-5* substituted for *bal*. In addition, *psi* replaces *pdx-1* as a marker for IV. *psi* (*protein-synthesis-inhibited*) is a readily-scored temperature-sensitive conditional mutant that does not grow or survive at 34°C although it is normal on minimal medium at 25°. *arg-5* is scored by transferring progeny to minimal medium at 25°C, *psi* by transfer to arginine-supplemented minimal at 34°. Ascospores must be germinated at 25°C.

multicent-5 differs from *multicent-4* only in having the *OY323* inversion present in linkage group I.

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. See *How to determine mating type*). The *at* mutant (*attenuated* morphology) is readily scorable on minimal (with or without supplements) at 2 or 3 days (34°C). Growth is flat on the surface, with scattered specks of conidiation. In *wc-1* (*white collar-1*), carotenoids are absent in mycelia but not in conidia. Scoring *wc-1* is better at 34°C than at 25°.

Germinants are incubated until conidia become orange, preferably under illumination. Scoring of *ylo-1* (*yellow-1*) is unreliable in young (3- or 4-day) cultures, especially in combination with *wc-1*, but it becomes increasingly clear with age. Thje color difference is more apparent under some light sources than

others, so if difficulty is experienced distinguishing *ylo* from the orange wild type, different fluorescent or other sources of illumination should be tried. *acr-2* is clearly resistant to 50 µg/ml acriflavine on solid medium. Small inocula should be used when scoring *pdx-1*.

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. Linkage, of course, is indicated if parental combinations are significantly more frequent than recombinants among the progeny. For ratios deviating significantly from 1:1, see table in Perkins (1944) or in *How to use genetic methods for detecting linkage*.

The new *multicent* testers are heterokaryon-compatible with strains of OR background (*het-C het-d; het-e; het-*x^{OR}). They are available as highly fertile phenotypically wild-type heterokaryons in combination with the inactive-mating-type *helper-1* strain (Table 1). Although the homokaryotic testers can be used for crossing, labor can be minimized by using a helper-assisted heterokaryon as female parent, improving fertility and making it unnecessary to supplement the crossing medium.

Figure 1, which is a tally sheet for scoring markers in crosses by *multicent-4*, can be adapted for use with other *multicent* testers.

References

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Table 1. Constitution of multicent testers 1 through 5

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

FGSC Numbers

Strain designation	***A***	***a***	***A* + *helper-1***	***a* + *helper-1***			
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			

Tally for Multicentromere Tester 4

Cross No._____ Strain or locus tested:_____

Parents: arg-5; acr-2^{R}; psi; at; ylo-1; wc-1; ____ X _____ ____

Linkage of unknown to markers (un = _____)

			at-un	wc-un	ylo-un	acr-un	arg-un	psi-un	mt-un
(P)	+	-							
(P)	-	+							
(R)	+	+				(P)			
(R)	-	-				(P)			

Figure 1.

How to use *multicent* strains for mapping genes and translocation breakpoints.