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Linkage testers for genes far from centromere

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Abstract

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Multiply marked strains now exist for efficient testing of linkage to the centromeres of all seven linkage groups in N. crassa (multicent A and a. N.N. 19: 33, 1972). These can be expected to reveal the linkage group of a substantial proportion of new point mutants, probably a majority. Multicent has been valuable also for determining the linkage groups of translocation breakpoints.

When a mutant fails to show linkage to any of the multicent markers, additional tests are then required with distal markers. One alternative is to use alcoy (Genetica 40: 247, 1969), which tests for linkage to markers part way out in the right arms of I, II, III, IV and V, and near centromere

in VIL. But the extremes are not marked, nor are the remaining unmarked arms adequately tested by alcoy. A second alternative is to use testers having point mutants marking both ends of the individual linkage groups. Attempts have been made to construct such testers for each of the seven groups, using the most distal practical gene markers (NN 19:30,1973; 20:40,1973). Only two of the 14 arms are tested by any one of these strains.

With the object of increasing efficiency, we have now devised a set of four testers which collectively include the most distal practical markers for 12 of the 14 arms. (IIIL and VL are not included because no distal gene markers are known in these arms, and the centromere markers acr-2 and at, which are present in multicent, already provide nearly the best available test.) The new tester stocks are shown in Table 1.

Table 1. Multiply marked stocks for testing linkage in distal regions

Tips tested	Markers	FGSC No.
IL; IIL; IVL	un-5 al-2; pi; cys-10 A	2922
	a	2923
	(al-2 also tests medial IR)	
IR; IVR; VR	un-18; mat; his-6 A	2944
	a	2945
IIR; IIIR; VIR	un-15; dow; trp-2 A	2926
	a	2927
VIL; VIIL; VIIR	chol-2; spco-4 wc nt A	2924
	a	2925
	(wc also tests VIIC)	
Note: Tests for IIIL and VL are provided by acr-2 (IIIC) and at (VC), which are present in multicent. The following stocks provide another alternative:		
IIIC; VC; IR	sc; lys-1; al-2 A	230
	a	231

The markers in these tester strains are not all ideal phenotypically with respect to female-fertility or rate of growth. But they have been chosen as the best prospects available, and have been combined with other markers in such a way that scoring should be feasible in all combinations. Three of the markers are nonconidiating morphological mutants (pi, IIL; mat, IVR; spco-4, VIIL). Three are heat-sensitive conditional lethals (un-5, IL; un-18, IR; un-15, IIR, scorable at 25° vs. 34°-39°. The latter two grow slowly even at the permissive temperature, 25°). Stocks containing these markers are best used as fertilizing parents in crosses.

trp-2 grows optimally on indole (0.01 mg/ml) or on tryptophan plus phenylalanine (each 0.2 mg/ml). lys-1 grows optimally when 0.5 mg/ml lysine is provided. cys-10 prefers GCP or other complete medium to minimal plus methionine. nt is best treated as a niacin mutant and supplemented with nicotinamide. chol-2 is scored without difficulty on minimal slants if inocula are small. wc does not develop orange pigment in mycelia at 34°. Isolation numbers for all markers can be found in the FGSC stock list.

There has not been time to gain experience with all the new testers, nor to determine their practicality or effectiveness in locating those unmapped mutants that show no linkage with multicent. Nevertheless, such testers are needed and it seems desirable to make them available for trial use. Reports of experience with them -- both favorable and adverse -- would be appreciated as would information on the discovery of other suitably located markers that might provide improved alternatives.

Probably neither the old nor the new testers are adequate to detect linkage at the ends of IIIL, VL or VIR. In the last resort, linkage near these tips could be tested using chromosome rearrangements having suitably placed breakpoints. T(IR→VL)AR190 or T(IR→VL)ALS182 and T(IR→VIR)NM103 or T(IVR→VIR)ALS159 are suitable to use for VL and VIR respectively, but no rearrangement is available yet for IIIL. Tests with terminal rearrangements are quite straightforward (Genetics 77: 459, 1974), though inefficient in that only one tip is tested at a time. For a map showing tip breakpoints and terminal markers see Fig. 5 in Perkins and Barry 1977 (Adv. Genet. 19, pp. 164-165). - - - Department of Biological Sciences, Stanford University, Stanford, CA 94305.