

## How to use *alcoy* for linkage group assignment.

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### Background

Rather than test an unmapped gene successively for linkage to markers in each of the seven linkage groups, it is advantageous to test all linkage groups in a single cross. Efficiency is greatly increased by using as tester a strain called *alcoy*, which contains three unlinked reciprocal translocations tagged with markers that can be conveniently scored by inspection, without transfer (Perkins 1964, 1966; Perkins *et al.* 1969). Because deficiencies are inviable, the only meiotic products to survive in crosses heterozygous for *alcoy* are those with a balanced genome (either translocation sequence or normal sequence). Thus only one-fourth of the ascospores are black. Tester strains were improved by tagging the remaining chromosome with a fourth marker (Perkins and Björkman 1979, Perkins 1991. For a diagram showing meiotic pairing and marker linkages, see Figure 1 (Fig. 9 in Perkins *et al.* 2001: <http://www.fgsc.net/2000compendium/2000compendA.html>).

*alcoy* is an acronym for three translocation-tagging mutant genes. The genotype of the *alcoy* testers is *T(IR;IIR)4637 al-1*; *T(IVR;VR)R2355, cot-1*; *T(IIIR;VIL)ylo-1*; *csp-2*. The markers are *albino-1*, *colonial temperature sensitive-1*, *yellow-1*, and *conidial separation-2* (which marks linkage group VII). All four are readily scored by eye. The strains grow on minimal medium. No transfer to test-media is necessary.

Crosses heterozygous for *alcoy* have proved useful in cytological studies of the synaptonemal complex and recombination nodules (Gillies 1972, 1979; Lu 1993). Their main application has been for genetic mapping, however.

In practice, about two-thirds of new point mutants show linkage to one or another of the four markers. Because each translocation-linked marker tags two chromosomes, a follow-up cross is needed for assignment of a gene to one of the two alternatives. For example, if a gene shows linkage to *cot-1* in the initial cross to *alcoy*, crossing it to the normal-sequence *cot-1*; *al-3* follow-up tester will show whether it is in linkage group IV or in V..

The *alcoy* testers have also proved useful in determining linkage of new translocations that have distal breakpoints (Perkins 1991). Normal-sequence follow-up testers with three or four linkage groups marked are also available for use when a new translocation shows linkage between two of the *alcoy* markers. New translocations that fail to link two of the *alcoy* markers are best mapped using a normal-sequence *multicent* tester. (See *How to use multiply marked multicent testers for mapping genes and translocation breakpoints.*)

### Procedure

Mature black ascospores from crosses made on slants of synthetic cross medium are isolated to minimal slants in 75 mm culture tubes and heatshocked 30 minutes in a 60°C water bath. After 3 days incubation at 34°C, *cot*<sup>+</sup> progeny will have grown up and conidiated while *cot*-minus progeny are barely visible as tiny colonies. Tubes that are not grown up (*cot*-minus and nongerminants) are moved to 25°C, where the *cot*-minus will grow up in 3 days and be ready for scoring the remaining markers. Appearance of the *yellow* marker varies with the type of illumination, and the pigment difference becomes more distinct as cultures age. *Conidial separation* is scored by tap-test of inverted tubes: Loose conidia on the tube wall are diagnostic of *csp*<sup>+</sup>. The *trp-1* marker in the follow-up testers is conveniently scored by blue fluorescence under long-wave UV when grown on indole-supplemented minimal medium.

Genotypes, FGSC Numbers, and linkage groups of *alcoy*; *csp-2* and of the follow-up testers are listed in Table 1.

Figure 2 is a convenient record-sheet for tabulating numbers when scoring progeny from a cross with *alcoy*; *csp-2*.

## References

Gillies, C.B. 1972. Reconstruction of the *Neurospora crassa* pachytene karyotype from serial sections of synaptonemal complexes. *Chromosoma* 36: 119-130.

Gillies, C. B. 1979. Relationship between synaptonemal complexes, recombination nodules and crossing over in *Neurospora crassa* bivalents and translocation quadrivalents. *Genetics* 91: 1-17.

Lu, B. C. 1993. Spreading the synaptonemal complex of *Neurospora crassa*. *Chromosoma* 102: 464-472.

Perkins, D. D. 1964. Multiple interchange stocks for linkage detection. *Neurospora Newslett.* 6: 22.

Perkins, D. D. 1966. Experience using *alcoy* multiple translocation tester strains to assign genes and chromosome rearrangements to linkage groups. *Neurospora Newslett.* 9: 11-12.

Perkins, D. D. 1991. *Neurospora alcoy* linkage tester stocks with group VII marked, and their use for mapping translocations. *Fungal Genet. Newslett.* 38: 83.

Perkins, D. D., and M. Björkman. 1979. Additional special purpose stocks. *Neurospora Newslett.* 26: 9-10.

Perkins, D. D., D. Newmeyer, C. W. Taylor, and D. C. Bennett . 1969. New markers and map sequences in *Neurospora crassa*, with a description of mapping by duplication coverage, and of multiple translocation stocks for testing linkage. *Genetica.* 40: 247-278.

Perkins, D. D., A. Radford, and M. S. Sachs. 2001. *The Neurospora Compendium: Chromosomal Loci.* Academic Press., San Diego.

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Table 1. Constitution of *alcoy*; *csp-2* and follow-up testers.

<u>Genotype</u>	<u>FGSC Numbers</u>	<u>Linkage Groups</u>
<i>alcoy</i> ; <i>csp-2</i>	3661 (A) 3434 (a)	T(I;II); T(IV;V); T(III;VI); VII
<i>al-1</i> ; <i>arg-5</i>	1205 (A) 1206 (a)	I; II
<i>al-1</i> ; <i>pe</i>	1203 (A) 1204 (a)	I; II
<i>trp-1</i> ; <i>ylo-1</i>	6808 (A) 1208 (a)	III; VI
<i>cot-1</i> ; <i>al-3</i>	3612 (A) 3613 (a)	IV; V
<i>cot-1</i> ; <i>inl</i>	1243 (A) 1244 (a)	IV; V
<i>al-1</i> ; <i>arg-5</i> ; <i>csp-2</i>	6664 (A) 6665 (a)	I; II; VII
<i>cot-1</i> ; <i>al-3</i> ; <i>csp-2</i>	6666 (A) 6667 (a)	IV; V; VII
<i>trp-1</i> ; <i>ylo-1</i> ; <i>csp-2</i>	6680 (A) 6681 (a)	III; VI; VII
<i>al-1</i> ; <i>arg-5</i> ; <i>cot-1</i> ; <i>inl</i>	1885 (A) 1886 (a)	I; II; IV; V
<i>al-1</i> ; <i>arg-5</i> ; <i>trp-1</i> ; <i>ylo-1</i>	2124 (A) 1888 (a)	I; II; III; VI

<i>trp-1; cot-1; inl; ylo-1</i>	1987 (A) 1988 (a)	III: IV: V: VI
<i>trp-1; cot-1; al-3; ylo-1</i>	4321 (A) 4322 (a)	III: IV: V: VI

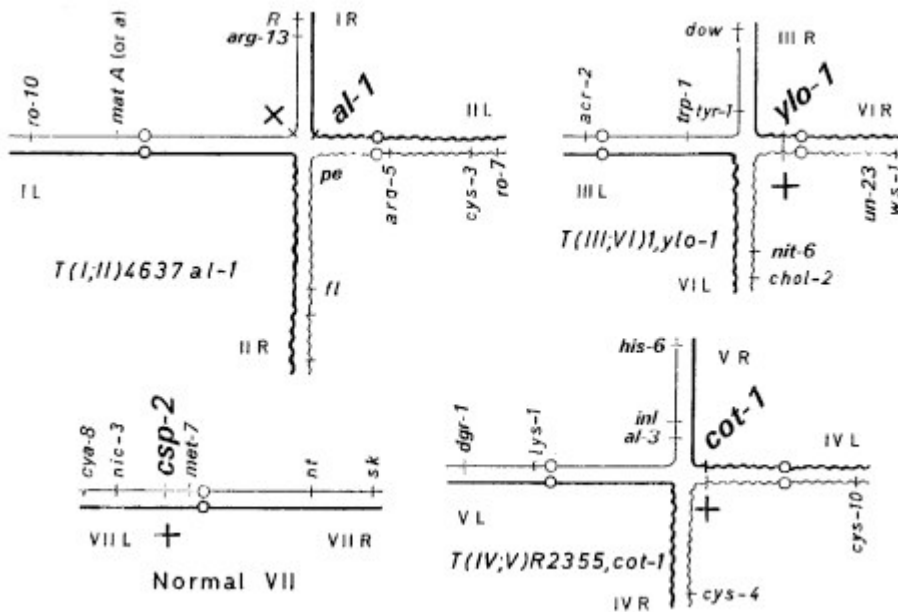


FIGURE 1. Linkage relations in a cross of the linkage tester *alcocy; csp-2* × *Normal sequence*. Chromosomes are shown paired as in meiotic prophase I. The *alcocy; csp-2* chromosomes are drawn as heavy lines, and those from the standard-sequence parent are shown as thin lines. Segments from odd-numbered linkage groups are shown as straight lines and even-numbered as wavy lines. The only mutant markers present in the *alcocy* parent are *al-1*, *cot-1*, *ylo-1*, and *csp-2*. These are shown in large letters. The *al-1* mutation is inseparable from the *T(I; II)4637* breakpoint in linkage group I, *cot-1* is a few units proximal from the *T(IV; V)R2355* breakpoint in IV, and *ylo-1* is a few units from the *T(III; VI)1* breakpoint in V (arbitrarily shown as centromere-proximal). Also shown are the locations of markers present in the standard-sequence follow-up testers, a few key markers for orientation, and some useful distal markers that are far removed from the breakpoints. The latter are used only when no linkage to the *alcocy* markers is detected. Interval lengths and relative distances are not to scale, but gene order is as shown. Copied from Perkins *et al.* (2001).

FIGURE 2. Record sheet for crosses of *alcocy; csp-2* × an unmapped mutant gene (“*unknown*”) or translocation.

[See PDF sent as aseparate attachment.]

**RECORD SHEET FOR CROSSES WITH alcov**

Date Crossed \_\_\_\_\_  
 Date heatshocked and put at 34° \_\_\_\_\_  
 Number isolated \_\_\_\_\_

CROSS NO. \_\_\_\_\_

TESTING MUTANT: \_\_\_\_\_

**I. got<sup>+</sup> (up after 3 days at 34°):**

Date sorted \_\_\_\_\_  
 Date scored for unknown \_\_\_\_\_  
 Date scored for al and ylo \_\_\_\_\_

csp <sup>-</sup> _____	}	unknown <sup>-</sup> _____	}	al <sup>-</sup> _____	}	ylo <sup>-</sup> _____
csp <sup>+</sup> _____				al <sup>+</sup> _____		ylo <sup>+</sup> _____
csp <sup>-</sup> _____	}	unknown <sup>+</sup> _____	}	al <sup>-</sup> _____	}	ylo <sup>-</sup> _____
csp <sup>+</sup> _____				al <sup>+</sup> _____		ylo <sup>+</sup> _____

**II. cot<sup>-</sup> (up after 5 days at 25°):**

Date moved from 34° to 25° \_\_\_\_\_  
 Date scored \_\_\_\_\_

csp <sup>-</sup> _____	}	unknown <sup>-</sup> _____	}	al <sup>-</sup> _____	}	ylo <sup>-</sup> _____
csp <sup>+</sup> _____				al <sup>+</sup> _____		ylo <sup>+</sup> _____
csp <sup>-</sup> _____	}	unknown <sup>+</sup> _____	}	al <sup>-</sup> _____	}	ylo <sup>-</sup> _____
csp <sup>+</sup> _____				al <sup>+</sup> _____		ylo <sup>+</sup> _____

Number grown \_\_\_\_\_  
 Non-germinants \_\_\_\_\_  
 % germination \_\_\_\_\_