How to use helper strains for maintaining and crossing handicapped recessive mutants, for forcing and resolving heterokaryons, and for determining heterokaryon compatibility.

Background

Mutant strains are often handicapped in various ways, manifested as sterility, slow growth, lack of conidia, poor viability, or instability (Barry 1992, Perkins 1986, Perkins 1993). Strains that do not conidiate are difficult to preserve, either on silica gel, by lyophylization, or by freezing. However, handicapped strains are readily handled and preserved in phenotypoically normal forced heterokaryons using a suitable marked partner. Helper strains that contain both a forcing nutritional marker and a mutation that inactivates mating type are convenient as partners. In *N. crassa*, the existing helpers are all in Oak Ridge genetic background. Thus, they are also useful for determining whether a marked strain is of Oak Ridge heterokaryon incompatibility genotype.

Helpers in *N. tetrasperma* (Perkins 1994) carry the gene *E*: *Eight spore*, which prevents the *E* component of a heterokaryon from contributing progeny when the strain being tested also carries the dominant *E* allele.

Procedure

N. crassa helpers. Visible fresh inocula of the two strains to be combined are superimposed at a spot on minimal medium, as is done when initiating any forced heterokaryon. Helpers carrying *mat* a^{m1} (Perkins 1984) or mat^{Δ} (*mat A* deletion) (Metzenberg and Sachs 2002) have lost both the ability to mate and the heterokaryon incompatibility that is specified by the mating-type genes. Strains with *mat* a^{m33}, which has lost only the *het*-incompatibility function, can also be used. Other helpers among those listed below are designed to enable the original sheltered strain to be extracted selectively from the heterokaryon as a pure homokaryotic culture, free of the helper.

When the strain to be sheltered grows well on minimal medium, a heterokaryon can neverthless be forced by using a helper that carries both a forcing nutritional marker and the dominant gene *Bml*, which confers Benomyl resistance. The heterokaryon is formed on minimal medium that contains Benomyl. (Wild type Neurospora is sensitive to Benomyl.) If, in addition, the helper component carries the herpes simplex thymidine kinase gene tk^{+}, the heterokaryon is unable to grow on 5-fluorouracil-2′-deoxyriboside (FUDR) (Sachs *et al.* 1997). The sheltered component can therefore be extracted in pure monokaryotic condition, free of helper nuclei, by transferring to medium containing FUDR (Metzenberg and Sachs 2002).

The a^{m1} *N. crassa* helper strains listed below are all Oak Ridge heterokaryon compatible. Strains to be sheltered must also be OR-compatible in order to form a heterokaryon. Any of these helpers can be used to determine whether a particular strain is OR-compatible, even if the mating type of the tested strain is not known.

mat a^{m1} *ad-3B cyh-1*	*helper-1*	FGSC 4564
mat^{Δ} tk^{+}(FUDR^{S}) *cyh-1; inl; Bml pan-2*	*helper-2*	FGSC 8745
mat^{Δ} *his-2* tk^{+}(FUDR^{S}) *cyh-1; inl; Bml pan-2*	*helper-4*	FGSC 8746
mat^{Δ} *his-3* tk^{+}(FUDR^{S}) hyg^{R} *cyh-1; Bml pan-2*	*helper-5*	FGSC 8747
mat^{Δ} *his-3 cyh-1; inl; Bml pan-2*	*helper-6*	FGSC 8748

N. tetrasperma helpers. Crosses heterozygous for the dominant *Eight-spore* gene *E* are fertile, but homozygous $E \times E$ crosses produce barren perithecia with few or no ascospores. Therefore, a marked monokaryotic *E* strain can be used as a helper by putting it into a forced heterokaryon with a

disadvantaged E^+ strain of the same mating type. When such a heterokaryon is crossed to any *E* strain of the opposite mating type, all progeny will be parented by the sheltered E^+ component (Perkins 1994). Marked strains suitable for use as helpers are *met(123) E A* (FGSC 7568), *lys(112) E a* (FGSC 7569). See the FGSC Stock list for alternative *E* strains that carry other forcing markers. These markers are in the genetic background of *N. tetrasperma* reference strains 85A and 85a.

References

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