

Methods for extracting markers from Spore killer strains of *Neurospora crassa*

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A mutant gene is most simply transferred from killer to nonkiller by crossing a marked killer strain x *Sk*-sensitive *acr-2R*, plating ascospores on medium supplemented with acriflavine, and recovering mutant progeny among the acriflavine-resistant colonies, which are nonkillers that presumably originated from rare heterozygous or heterokaryotic ascospores.

In the course of experiments with *Sk-2K* and *Sk-3K*, mutations unrelated to Spore killer have occasionally occurred in Spore killer strains. It has sometimes been desirable to transfer the new mutant alleles into ordinary nonkiller strains. This cannot be done simply by crossing to laboratory wild types, which are all *Sk*-sensitive nonkillers, because in crosses of *killer* x *sensitive*, essentially all of the progeny are killers. For a summary description of Spore killers, see Turner and Perkins 1991 *Am. Naturalist* **137**:737-748 or Turner *et al.* 1987 *Fungal Genet. Newsl.* **34**:59-62.

Three methods are available for transferring a marker out of a killer strain. Two of the methods depend on the fact that sensitive meiotic products from a cross of *killer* x *sensitive* are rescued if a sensitive nucleus is included in the same ascospore wall with a killer nucleus. The third method employs genes that confer resistance to killing.

The preferred method makes use of an *Sk*-linked selectable marker, *acr-2*. The *acr-2* locus, which is near the centromere of linkage group III, is included in the 30-unit long *Sk* complex. The mutant *acr-2R* allele, which confers resistance to acriflavine, shows no or little (10^{-5}) recombination with *Sk-2K* or *Sk-3K*. Killer strains contain the wild type *acr-2S* allele, which renders them vegetatively acriflavine-sensitive. To extract a marker, the marked killer strain is crossed by a killer-sensitive *acr-2R* strain. Allele KH5 is recommended (FGSC 4043 A or 4044 a). Ascospores are plated on a medium containing acriflavine (50 g/ml) and colonies are picked. Acriflavine-resistant survivors, which are nonkillers, occur at a frequency of about 10^{-3} (Campbell and Turner 1987 *Genome* **29**:129-135; DP unpublished crosses). These originate from heterokaryotic ascospores or from disomics of constitution (*acr-2S* killer)/(*acr-2R* nonkiller) (Campbell and Turner 1987). The disomics break down rapidly, producing an *acr-2R* nonkiller derivative. Half of the acriflavine-resistant survivors are expected to contain the mutant marker if it is unlinked to *Sk*, and fewer if it is *Sk*-linked.

We have found the acriflavine-resistance method to be simple and effective. Extraction of a new *frost* allele, P4405, provides an example. This originated as a spontaneous mutation in *Sk-2K*. Eight colonies appeared following heatshock when a dense suspension of ascospores from *Sk-2K fr* x *acr-2R* was spread on a single plate of minimal sorbose + acriflavine. The *acr-2R* progeny were *Sk*-sensitive, and five of them were *fr*.

A second method for extracting markers from a Spore killer strain is to employ an ascus-development mutant that acts to enclose killer and nonkiller nuclei in the same ascospore wall. The *Sk*-sensitive component, which survives, is extracted from a heterokaryotic germling by streaking or plating conidia. We have used the ascus-dominant mutant *Banana*, which produces a single giant ascospore that encloses the contents of an entire ascus (Raju and Newmeyer 1977 Exp. Mycol. **1**:151-165). As an example, this method was used by Virginia Pollard to extract a new pigment mutation, *vivid* (P4246), from the *Sk*-2K strain in which it originated. *vvd* was obtained free of the killer factor by crossing it by *Banana*, germinating giant ascospores, and plating conidia from one of the heterokaryotic germinants on sorbose-minimal medium. Among 13 *vivid* cultures of single-conidial origin, six were *Ban*⁺ nonkillers. This method, while effective, is more laborious than the acriflavine-resistance method because it requires conidial platings and additional progeny tests.

A third possible method for extracting mutants from a Spore killer strain would be to cross the mutant *Sk* strain by a nonkiller strain that contains a gene such as *r(Sk-2)-1*, which confers resistance to killing on progeny that are nonkillers. *r(Sk-2)* and *r(Sk-3)* strains that might be used are listed by Turner and Perkins 1993 Fungal Genet. Newsl. **40**:76-78. A potential disadvantage is that the known *r(Sk)* genes, which all originated in wild-collected isolates, have not been extensively inbred to our standard Oak Ridge wild type. We have not used this method.

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