How to determine whether a gene is essential for survival.
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Background

Whether a gene function is essential can be determined by inactivating the gene and showing that the nonfunctional allele acts as a recessive lethal. The inactivated gene is sheltered by complementation in a heterokaryon. If the gene is essential, only the nonmutant component of the heterokaryon can be recovered, either vegetatively or in progeny from a cross. Inactivation can be accomplished by repeat induced point mutation (RIP) (reviewed by Selker 2002) or by targeted gene replacement.(Ninomiya *et al.* 2004).

Inactivation by RIP

This involves crossing a strain that contains a duplicate copy of the gene of interest and recovering progeny that have undergone RIP. The duplication parent is readily obtained by ectopic integration following transformation.

Two ways have been described for obtaining heterokaryotic single-ascospore progeny. 'Sheltered disruption' (Metzenberg and Groteluechen 1992) and 'Rip & Rescue' (Ferea and Bowman 1996). Sheltered disruption uses as one parent a strain that generates disomic meiotic products. These promptly break down to form the required heterokaryon. Rip & Rescue uses crosses in which both parent strains contain extra copies of the gene of interest at ectopic positions. Progeny are then selected in which one ectopic copy is still active while the native gene has undergone RIP.

Targeted gene inactivation.

Replacement is accomplished with 100% efficiency by using a PCR-based method devised by Ninomiya *et al.* (2004). Electroporation is used to introduce transforming DNA into a recipient strain that is deficient for nonhomologous end-joining. Insertion is therefore restricted to the homologous locus. When transformation occurs, a heterokaryon is produced that contains both transformed and nontransformed nuclei, and there is no need to make a cross.

Procedure

Sheltered Disruption

See (Metzenberg and Groteluechen 1992) and FGSC Catalog, Part VI. I, for the kit of strains designed to be used in this method. See Harkness *et al.* (1995) and Nargang *et al.* (1995) for applications of sheltered RIP, with diagrams and details.

If growth of a null mutant obtained by this method is severely limited rather than being eliminated completely, the phenotype of the sublethal mutant can be examined by using gradient plates and streaking them with conidia of the specially designed heterokaryon that incorporates the mutation together with diagnostic antibiotic-resistance markers (Metzenberg and Groteluechen 1992)..

Rip & Rescue.

See Ferea and Bowman (1996) for use of this method, with diagrams and details.

Targeted disruption.

See Ninomiya *et al.* (2004) for a description of the method and of the key strains *mus-51* and *mus-52*, which are deficient for nonhomologous end-joining and which can be used to obtain 100% homologous integration. Stocks are available (*mus-52::Hyg*^{r}, FGSC 9567 *A*, 9568 *a*, and *mus-51::Hyg*^{r}, FGSC 9595 *A*).

Targeted disruption is being used by the Neurospora Genome Project http://www.dartmouth.edu/%7Eneurosporagenome/proj_overview.html (Colot *et al.*, in preparation). Knockouts and knockout cassettes will be available through FGSC.

References

Colot, H. V., G. Park, G. E. Turner, C. Ringelberg, C. M. Crew, L. Litvinkova, R. L. Weiss, K. A. Borkovich, and J. C. Dunlap. Overlapping functions for multiple novel transcription factors in Neuorspora. (In preparation.)

Ferea, T. L., and B. J. Bowman. 1996. The vacuolar ATPase of *Neurospora crassa* is indispensable: Inactivation of the *vma-1* gene by repeat-induced point mutation. Genetics 143: 147-154.

Fungal Genetics Stock Center. 2004. *Catalogue of Strains, 10th Edition.* (Part VI. I: Strains for disruption of essential genes.)

Harkness, T. A. A. , R. A. Rothery, J. H. Weiner, S. Werner, J. E. Azevedo, A. Videira, and F. E. Nargang. 1995. Disruption of the gene encoding the 78-kilodalton subunit of the peripheral arm of complex I in *Neurospora crassa* by repeat induced point mutation (RIP). Curr. Genet. 27: 339-350.

Metzenberg, R. L., and J. S. Grotelueschen. 1992. Disruption of essential genes in Neurospora by RIP. Fungal Genet. Newslett. 39: 37-49.

Nargang, F. E. , K. P. Künkele, A. Mayer, R. G. Ritzel, W. Neupert, and R. Lill. 1995. 'Sheltered disruption' of *Neurospora crassa* MOM22, an essential component of the mitochondrial protein import complex. EMBO J. 14: 1099-1108.

Neurospora Genome Project. 2004. http://www.dartmouth.edu/%7Eneurosporagenome/proj_overview.html

Ninomiya, Y., K. Suzuki, C. Ishii, and H. Inoue. 2004. Highly efficient gene replacements in Neurospora strains deficient for nonhomologous end-joining. Proc. Nat. Acad. Sci. USA 2004. 101: 12248-12253.

Selker, E. U. 2002. Repeat-induced gene silencing in fungi. Adv. Genet. 46: 439-450.

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