

## How to inbreed for increased isogenicity.

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### Background

For many investigations, uniformity of genetic background is important. This is best accomplished for each species by setting up a well-endowed representative strain for reference. This strain is then used as a source of new mutations and as recurrent parent in backcrosses when mutations are introduced from other strains. The standard of choice for *N. crassa* is OR23-1V A (FGSC 2489), which was the source of DNA used for sequencing the genome (Galagan *et al.* 2003). The *mat a* counterpart, ORS-6a (FGSC 4200), is a product of at least 13 generations of backcrosses to OR23-1VA (Mylyk *et al.* 1974, Newmeyer *et al.* 1987). These standards will be referred to as Oak Ridge (OR) wild types. The standard *fluffy* (OR) testers (Perkins *et al.* 1989) are highly inbred to the OR standards.

Polymorphisms are present in many laboratory stocks. Although many of the mutant alleles in FGSC originated in OR genetic background, a large number of useful markers originated in other backgrounds or acquired a mixed background by being intercrossed with other strains. Even when mutations were induced in the standard OR background, additional cryptic differences may have originated during mutagenesis or may have arisen spontaneously.

Strains of *N. crassa* from nature are highly polymorphic for *het* genes (Mylyk 1976), and commonly used laboratory strains differ at several *het* loci. (See *How to identify and score genes that confer vegetative (heterokaryon) incompatibility*.) A common objective of backcrossing is to attain *het*-compatibility with OR.

The *het*-genotype of OR wild types and derivatives thereof is *het-C*, *het-d*, *het-e*, *het-i*, *het-5<sup>OR</sup>*, *het-n<sup>OR</sup>*, where *n* represents any number greater than 5. (Letters rather than numbers were used when the first four *het* genes were discovered and named, with upper and lower case being used to distinguish alternate alleles, This unconventional usage has been retained for *het-C*, *-D*, *-E*, and *-I*. However, numbers have been used for later-discovered *het* genes, beginning with *het-5*. The *het-C* allele has sometimes been called *het-c<sup>OR</sup>* or *het-C<sup>OR</sup>*.) Recurrent backcrosses will of course be needed to attain uniformity of background when genes are introduced from one species or wild population into another. The question arises, how many generations will be required to achieve the desired degree of isogenicity?

One might jump to the conclusion that seven generations of backcrossing is sufficient to assure uniformity at the 5% confidence level. While a value of  $(1/2)^7$  would be correct for the probability of retaining heterozygosity at a single locus after seven generations, this is far from true for the entire genome, where genome size, chromosome number, and recombination frequency all contribute to retarding the attainment of isogenicity.

Heterogeneity persists mostly in tracts of various length.

In a heterothallic fungus such as *N. crassa*, heterozygosity at the mating type locus is compulsory for crossing, while crossing over creates patches in the autosomes that are of independent origin ('allogenic'; heterozygous in crosses). Leslie (1981) has made a rigorous analysis, providing formulas for calculating the fraction of the genome expected to have been rendered homozygous in each recurrent generation. Thus, even after 10 generations of recurrent backcrossing to the same mating type in *N. crassa*, 2% of the genome is likely to remain allogenic. More than 95% of this diversity is accounted for by a 20 map-unit long allogenic tract containing the mating type locus.

The downside to inbreeding in a heterothallic species such as *N. crassa* is that genes leading to ascus abortion become fixed. Consequently, 'bubble asci' with eight tiny aborted ascospores are abundant in crosses between the *mat A* and *mat a* OR standards or between strains that are highly inbred to them (Raju *et al.* 1987). Because bubble-ascus abortion is nonselective with respect to ascospore genotype, genetic ratios are not affected in the surviving progeny. There is no shortage of mature viable asci in bubble-ascus crosses, and occurrence of bubble asci does not affect genetic analysis. The bubble-ascus phenomenon can therefore be ignored for most purposes.

## Procedure

When backcrossing is to a fixed parent, progress toward isogenicity is most rapid in early generations. A single cross may be enough to obtain progeny that are heterokaryon-compatible. Two or three generations of backcrossing may suffice to rid a new mutation of major unwanted background differences that were acquired during mutagenesis. Many more generations will be required when genes are being introgressed from another species. Careful quantitative studies of recombination or gene expression will also dictate that strains be backcrossed extensively. Leslie's formulas can be used to estimate progress toward isogenicity at each generation.

The labor involved in backcrossing is minimal. Elapsed time is the main problem. Generation time can be reduced to less than 3 weeks by having plates or slants of the recurrent parent ready to fertilize as soon as progeny of each generation are available.

The convention for recurrent crosses to the same standard strain is to call haploid progeny of the first generation 'f<sub>1</sub>'. Progeny of f<sub>1</sub> x standard are called b<sub>1</sub>; progeny of b<sub>1</sub> x standard, b<sub>2</sub>, etc.

## References

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