How to use RFLP for mapping cloned genes.

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Background

Restriction fragment length polymorphisms (RFLPs) can be used to determine the approximate map location of any cloned piece of DNA. Although unlikely to be needed routinely, now that the genome sequence is available, situations may arise where RFLP mapping will be useful. To establish an RFLP mapping system for *N. crassa*, R. L. Metzenberg and coworkers crossed strains with multiple sequence differences, an Oak Ridge laboratory strain (designated "O") and a Mauriceville-1c wild-collected strain (designated "M") (Metzenberg et al. 1984 Neurospora Newsl. 31:35-39; ibid. 1985 Proc. Natl. Acad. Sci. U.S.A. 82:2067-2071). Progeny from two separate crosses have been widely distributed and used for mapping. For the first cross, 38 progeny from 18 ordered asci were analyzed. Because nonsister spores from the same half of the ascus were selected, first-division segregation can be distinguished from second-division. For the second cross, 18 random ascospore progeny were analyzed. For several reasons, the first cross is preferred for RFLP mapping. Resolution is better because more loci have been scored; distance from the centromere can be estimated regardless of which linkage group is involved; double crossovers within intervals can be recognized as can putative gene conversions and scoring errors (see Nelson and Perkins 2000). The most recent compilation of results was by Nelson and Perkins (2000) and their tables were reproduced with an expanded explanatory introduction as Appendix 3 in Perkins *et al.* (2001). The first column of the tables shows loci ordered as in a genetic map.

Procedure

Progeny from Metzenberg's two crosses are available from FGSC (2004). The unmapped probe of interest is used to score each of the 38 (or 18) isolates as "O" or "M". The tables are then scanned to find the mapped locus (or neighboring loci) that most closely resembles the unmapped segment in its pattern of "O" and "M" markers. The following details are from Metzenberg *et al.* (1984):

- *Steps that need only be done once in each lab*: Grow the set of parental and progeny strains (from FGSC) from one of both of the two crosses. The strains are grown at 25°C in Vogel's medium with 2% sucrose, supplemented in the first cross with 2 mM L-arginine and 50 µg/ml inositol, and for the second cross with 1 mM L-citrulline, 1 mM uridine, 1 mM L-lysine, 50 µg/ml inositol, and 2 µg/ml each of thiamine HCl and nicotinamide. (Citrulline rather than arginine is used to avoid competition with lysine for uptake.)
- *Steps that need to be done for each cloned DNA segment that is to be mapped*:
  1. Prepare plasmid or phage DNA carrying the segment and label it radioactively by nick translation or other means.
  2. Prepare digests of DNA from the two parental strains of a cross with several arbitrarily chosen restriction enzymes to find one that will show a usable RFLP.
  3. Prepare Southern blots from agarose gel electrophoresis of those digests and probe.
  4. Choose the best enzyme, digest an aliquot of DNA from progeny as well as from parentals, blot, probe and score. (In practice, steps 2 and 3 are often skipped and one or

more enzymes are chosen to try simultaneously with parentals and progeny. The amount of polymorphism is large enough so that this is a reasonably efficient strategy.)

References

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