Feher, Zs.

Construction of a Neurospora crassa gene library in a cosmid vector.

For cloning specific genes from the genome of an organism it is advantageous to use a cosmid with which it is possible to cover the genome with a relatively small number of clones via selective cloning of large pieces of DNA. For the construction of a N. crassa gene library we chose the yeast cosmid BHB 3030 (a kind gift of Hinnen, A.), which also enables us to test the expression of the recombinant cosmids in yeast. This 10.3 kb cosmid is a derivative of YEp6 (Struhl et al., 1979 Proc. Nat. Acad. Sci. USA 76: 1035-1039) and contains a fragment of 2 μ plasmid, the yeast his3 gene, ampicillin and tetracycline resistance genes (Ap^R: Tc^R), the cos region of phage λ and unique sites for restriction enzymes BamHI, SalI, BstEII and XhoI.

We used the N. crassa slime mutant (FGSC #1118) as a source of donor DNA. The high molecular weight DNA was prepared according to Kingsman et al, (1979 Gene 7: 141-151) with some modifications. 200 μg of N. crassa DNA was partially digested with MboI and size-fractionated on a 10-40% sucrose gradient (Maniatis et al., 1978 Cell 15: 687-701). 0.5 ml fractions were collected and checked for size by agarose gel-electrophoresis using the 33.5 kb XhoI fragment and the 27.5 kb HindIII fragments of λ DNA as markers. The fractions containing DNA fragments 30-40 kb in size were precipitated with ethanol. 3 μq of sized N. crassa DNA was ligated for 16 h at 14° C in a total volume of 10 μl to 2 μg of cosmid vector BHB 3030, linearized by BamHI. The ligated DNA was packaged in vitro into phage λ (Hohn, 1979 in Methods in Enzymology 68: 299-309, Academic Press, NY) and the phage particles were transduced into E. coli HB 101. The efficiency of the transduction was about 5000 Ap^R colonies/μg of donor DNA. 5000 colonies were picked and grown independently in microtiter dishes in YTB medium containing 100 μg/ml ampicillin. Glycerol was added to a final concentration of 20% and the gene library was stored at -20° C.

80% of the clones proved to be recombinant, as judged by the insertional inactivation of the Tc^R gene. Restriction analysis of cosmids from 24 random clones supported this result. The genome size of N. crassa is known to be about 27,000 kb (Krumlauf et al., 1979 Biochemistry 18: 3705-3713). As the average insert size in the gene library is estimated to be 30 kb, 900 clones correspond to one genome equivalent. Our 4000 recombinants cover the genome more than four times, this means that we have any N. crassa sequences represented with a probability of 98%, at least (Clarke et al., 1979 Cell 9: 91-99). Using the recombinant cosmids of this gene library one can try to get complementation of (1) E. coli, (2) yeast, (3) N. crassa auxotrophic mutants in addition to the possibility of screening by hybridization. The library may be useful for cloning directly nonselectable genes adjacent to selectable ones on large pieces of DNA and allows the application of "chromosomal walking" in N. crassa. - - - Institute of Biology, University Medical School, H-4012 Debrecen, Hungary.