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Abstract

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In microorganisms, genes can often be cloned directly by complementation of mutants with a genomic library. However, when one is searching for non-selectable genes (such as the Neurospora photomorphogenesis genes, which we have been transformants that make the number of transformants that must be screened make the identification of the gene tedious and time consuming. Here we describe a strategy that reduces the complexity of a Neurospora library to a limited number of clones that can then be screened by transformation, taking advantage of the following:

1. A large number of Neurospora genes have been mapped to the seven linkage groups (Perkins et al. 1982 Microbiol. Rev. 46:426-570).
2. Neurospora chromosomes can be purified by alternating-field gel electrophoresis (Orbach et al. 1988 Mol. Cell. Biol. 8:1469-1473; our results).

We purified and labelled a chromosome to serve as a probe for identifying specific clones from the Vollmer-Yanofsky cosmid library (Vollmer and Yanofsky 1986 Proc. Natl. Acad. Sci. USA 83:4869-4873). N. crassa strain T(T54M50), with a translocation of most of the left arm of chromosome VII to chromosome IV (Perkins et al. op. cit.) was used as the source of the chromosomes. The truncated chromosome VII (about 2.5 megabases) was separated from the other chromosomes using a contour-clamped homogenous electric field gel apparatus (Chu et al. 1986 Science 234:1582-1585) with a switching time of 60 min, at 2.5 Volts/cm for seven days at 6°C. About 4 micrograms of chromosome VII were recovered by electroelution of the chromosome band from five preparative agarose gels. Two micrograms of chromosome VII were labelled by nick-translation (using 1 uCl of [^32 P]dATP) and used to probe the 3072 cosmids of the ordered Vollmer-Yanofsky library on Hybond N filters.

The autoradiograms showed 255 positive clones of various intensities (from strong to weak signals). We removed from the list of positive clones (Table 1) 46 corresponding to the rDNA cluster (represented in this library by 64 cosmids) which lies on chromosome V. Since the rDNA is the major repetitive DNA of Neurospora, it must represent the major contaminant of our chromosome VII preparation. When our probe was hybridized with separated nitrocellulose-blotted Neurospora chromosomes, only chromosomes VII and V were detected, suggesting that any contamination of our preparation by sequences other than the rDNA must be minor.

To determine if our assignment of cosmids to chromosome VII was accurate, we compared our positive clones with those shown to map on chromosome VII by other groups. J. Dunlap told us of 6 cosmids mapping in the frq region, T. Schmidhauser of 7 cosmids from the un-10 region, and R. Geever of 19 cosmids from the qa cluster. Of these 32 cosmids, 24 (75%) were identified by our chromosome VII probe. Also, no false positives have yet been identified, either by comparing our list with that available from the Fungal Genetics Stock Center or from personal communication of groups working on N. crassa.

There are several possible explanations for the lack of positive signals with cosmids mapping on the right arm of chromosome VII and also for the observed differences in hybridization intensities. A trivial explanation is a difference in bacterial growth on the filters. Alternatively, the chromosomal DNA might not have been uniformly labelled. A third explanation is suggested by the reduced frequency of positive clones, 12 out of 21 (57%) among another series of homologous cosmids thought to map on chromosome VII (identified by T. Schmidhauser). This observation suggests that, in the case of a highly represented clone in the library, the probe can become so diluted that only a weak signal or no signal at all can be detected. We suggest that a more complete list of the cosmids mapping on the right arm of chromosome VII might be obtained by labelling the cosmid inserts that we and others have identified and using them to probe the library.

We would be grateful for any further information about chromosome VII-specific clones (including any knowledge of false-positive clones).

Table 1. Cosmids mapping on chromosome VII of T(T54M50)*

1:3F	1:4B	1:6G	1:7G	1:8F	1:8G	1:9A	1:12B	1:12D
1:12E	2:2H	2:5C	2:9A	2:10A	2:12F	3:2G	3:10F	3:10G
3:12B	4:5G	4:8E	4:9B	4:11H	4:12C	4:12H	5:1E	5:2E
5:9A	5:9H	5:12F	6:1A	6:1B	6:1C	6:1F	6:4F	6:10B
7:4G	7:6H	7:7B	7:9H	7:10A	8:5A	8:7D	8:8A	8:9B
8:11G	8:12H	9:1A	9:6E	9:7E	9:12A	9:12G	10:2C	10:3B
10:4F	10:5D	11:2D	11:3B	11:4G	11:5G	11:6A	11:8E	11:8H
11:10B	11:11A	12:2H	12:7G	12:10B	13:1B	13:2G	13:3A	13:4A
13:4H	13:7G	13:9A	13:9B	13:9C	13:10A	14:2H	14:3A	14:6A
14:9G	15:6H	15:7E	15:10B	15:10H	16:1B	16:1E	16:1G	16:3F
16:4B	16:9G	16:10H	16:12A	17:1C	17:3H	17:4E	17:4H	17:5F
17:5H	17:6H	17:7B	17:11H	18:1C	18:3A	18:4B	18:7C	18:7D
18:7H	18:8B	18:8H	18:10C	18:10D	19:3B	19:3D	19:6D	19:7D
19:8B	19:10E	19:12A	20:1c	20:1G	20:7E	20:8C	20:9H	21:4F
21:5F	21:7B	21:11B	21:11B	21:12F	22:2B	22:2C	22:3F	22:4G
22:5H	22:9F	23:1B	23:1C	23:2D	23:3C	23:9F	23:12C	23:12F
24:2B	24:5F	24:11E	25:1D	25:1E	25:1H	25:2B	25:3D	25:3E
25:5D	25:5B	25:5F	25:11C	25:11D	26:1H	26:2A	26:2B	26:3B
26:6D	26:6G	26:7F	26:7H	26:12B	27:1C	27:1E	27:5E	27:7F
27:9H	27:11H	27:12F	27:12G	27:12H	28:1C	28:2H	28:3F	28:3H
28:6B	28:6H	29:1C	29:1F	29:3F	29:5A	29:8H	29:12B	30:1A
30:8B	30:9B	30:10H	30:11C	31:1B	31:1D	31:2H	31:3F	31:3G
31:5c	31:8C	31:l11D	31:12D	31:12B	31:12H	32:4A	32:5H	32:7C
32:8E	32:10C							

* The number of positive signals found in adjacent wells was higher than would be expected for a random distribution of positive clones, and may reflect some cross-contamination that could have occurred at any time during the preparation of the library. If this is the case, the number of positive clones would be reduced accordingly.

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