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Abstract

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Colony hybridization has proven to be useful procedure for efficiently screening bacterial clones by RNA-DNA or DNA-DNA hybridization (Grunstein and Hogness, 1975 Proc. Natl. Acad. Sci. USA 72: 3961-3965). Such a technique would also be useful in Neurospora for the detection of specific DNA sequences, as in the case of integrated vector sequences in a transformant. The following protocol, which is a modification of the procedure used in yeast (Hinen, et at., 1978 Proc. Natl. Acad. Sci. USA 75: 1929-1933), allows for colony hybridization to be carried out in Neurospora.

S & S BA85 nitrocellulose filters were autoclaved and then dipped into Fries medium containing 1.8% sorbose. The moistened filters were placed on agar plates of the same medium that had been overlayed with l-2 ml of liquid medium. Droplets of conidial suspension from each strain to be tested were then spotted on the filters. The inoculated filters were incubated for 24 h at 30° C. The filters were then air-dried briefly and placed, in the following sequence, on Whatman 3 MM paper saturated with: (1) M sorbitol, 40 mM EDTA (pH 8.0), 50 mM DTT, 15 min, 30° C; (2) 1 M sorbitol, 40 mM EDTA (pH 8.0), 10% glusulase (Endo Labs., 90,000 units/ml), 3-4 h, at 37° C; (3) 0.5 M NaOH, 10x SSC, 8 min; (4) 0.5 M Tris-HCl (pH 7.4), 10x SSC, 4 min; and (5) 0.5 M Tris-HCl (pH 7.4), 10x SSC, 4 min. The filters were subsequently transferred to a S & S filtration block (#MV082) and, with suction applied, 200 ml of 3x SSC and 100 ml of chloroform were poured through the filter. Following air-drying the filters ere baked in a vacuum oven at 80° C for 2 h.

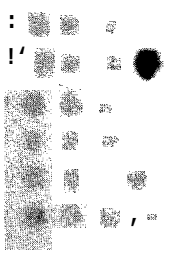

Figure 1. -- Example of the colony hybridization technique as applied to a set of Neurospora transformants.

The baked filters were soaked in 3x SSC for 15 min followed by 2 h in 3x SSC, 10x Denhardt's sol., 0.1% SDS at 65° C. Prehybridization was in 3x SSC, 10x Denhardt's sol., 0.1% SDS and 1 mg/ml salmon sperm DNA for 12 h at 65° C. Hybridization was carried out for 24 h at 65° C followed by washes in 3x SSC, 2x SSC, and lx SSC. For autoradiography Kodak XAR-5 film and DuPont Cronex intensifying screens were used. A light background was usually present in the autoradiographs on all of the colonies but did not interfere with scoring a positive response to the probe.

Figure 1 shows a colony hybridization screen of some homokaryotic Neurospora qa-2$^+$ transformants. The probe was 32P-labeled pBR322. A single colony has given a positive response indicating that at least part of the vector (pBR322) has integrated along with the qa-2$^+$ gene in this transformant.

After perfection of the technique described above, we learned that Stohl and Lambowitz (1983 Anal. Biochem. 134: 82) had independently developed a colony hybridization procedure with some similar features. The protocol we use follows the yeast colony hybridization procedure fairly closely with the exception of a longer enzyme treatment at a higher concentration and a more thorough washing of the nitrocellulose filters utilizing a S & S filtration block. We have not yet done an experimental comparison with the Stohl and Lambowitz procedure, but it appears from their data that the results the techniques provide are similar. Our experiences with colony hybridization, as presented here, provide additional information which may be helpful.

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