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Modified dot-blot hybridization technique for filamentous fungi.

Colony hybridization (Grunstein & Hogness, 1975 Proc. Nat. Acad. Sci. USA 72:3961-3965) allows the rapid screening of multiple strains for the presence or absence of particular DNA sequences. Two similar protocols have been developed for colony hybridization in Neurospora crassa (Stohl & Lambowitz. 1983 Anal. Biochem. 134:82-85; Paietta & Marzluf. 1984 Neurospora Newsletter 31:40). Both entail the growth of colonies on cellulose nitrate filters. We have analyzed strains of Neurospora transformed to benomyl resistance (Vollmer & Yanofsky, 1986 Proc. Nat. Acad. Sci. USA 83:4869-4873) by this method and have found it difficult to obtain repeatably uniform colonial growth of transformed strains on filters. We therefore eliminated this step, instead using liquid suspensions of cultures grown on nutrient agar slants as the source material for DNA extraction, immobilization and analysis by hybridization. The following is a detailed and annotated DNA dot-blotting protocol:

1. Resuspend a culture grown on an agar slant in 5 ml sterile HOH. These resuspended cultures are heterogenous, which will contribute to variability among replicates. Further, the density of these resuspensions will obviously vary with the state of the conidial slant. For more accurate quantitation one might adjust different resuspensions to constant optical density.

2. Add 0.5 ml of this suspension to a microfuge tube, pellet and decant. This volume of cell suspension can be reduced substantially (to 0.1 ml) and lower volumes may actually result in less non-specific hybridization (trapping).

3. Resuspend the pellet in 0.5 ml of 1 M sorbitol containing 1 mg/ml Novozym 234 (Novo Industries, Copenhagen). Incubate at 30° C for 60 min. Spheroplasting increases the hybridization signal.

4. Pellet, decant and resuspend in 0.4 ml of 0.4 M NaOH (Reed & Mann, 1985 Nucleic Acids Res. 13:7207-7221).

5. Filter onto a pre-wetted (first in water and then in 0.4 M NaOH) nylon membrane (Biotrans, ICN, Irvine, CA) in a dot-blot manifold (Schleicher & Schuell, Keene NH). In our experience, pelleting the cellular debris by centrifugation and filtering only the supernatant reduces background and speeds the filtering without any loss in hybridization signal.

6. Rinse each well with 0.4 ml of 2X SSC (Maniatis et al., 1982 Cold Spring Harbor Press)

7. Bake the membrane for 1 h at 80° C under vacuum.

8. Pre-hybridize, hybridize and wash normally. We pre-hybridize and hybridize in Blotto-formamide (Johnson et al., 1984 Gene Anal. Tech. 1:3-8) at 42° C. Washing the baked membrane in 2X SSC prior to pre-hybridization does not particularly affect the signal but may decrease background. Background can further be reduced by gently rubbing the membrane in 2X SSC, 0.1% SDS prior to pre-hybridization. Both of these steps probably reduce cellular debris which may trap labelled DNA non-specifically. Problems with background can be further alleviated by loading dilutions of the cleared supernatant from step 5. Negative (untransformed) and positive controls are useful for comparison.

We have used this protocol to analyze heterokaryotic transformants and cross progeny for the presence of pBR322-hybridizing sequences. The advantages of the method are that: (1) it obviates problems associated with disparate growth rates among strains to be examined, which is frequently the case with transformants, and (2) starting from a conidial slant the entire procedure including preparation, blotting, hybridization and washing can be completed within 24 h. Crowley & Oliver (1987 Exptl. Mycol. 11:70-73) have reported a similar method in which cells are grown and spheroplasted in the wells of microtiter dishes. - - - Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

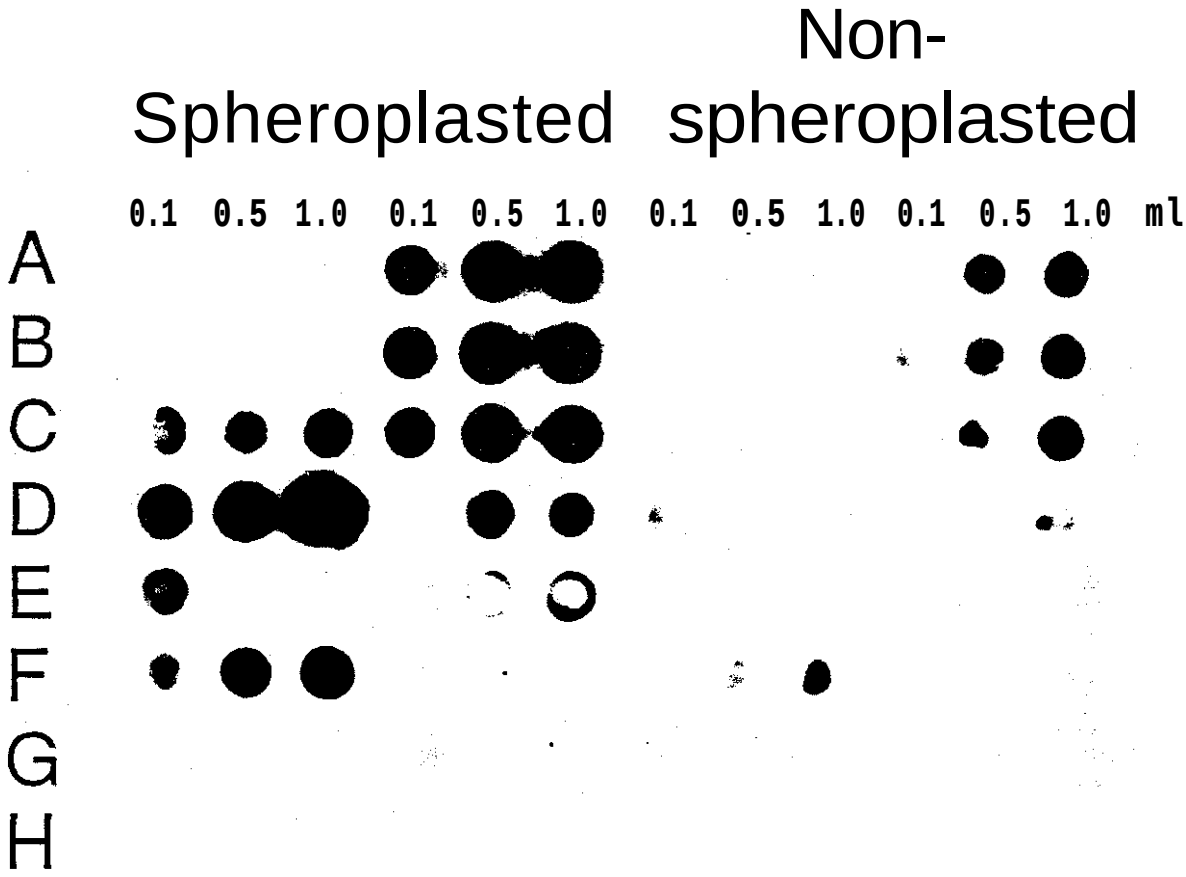

Figure 1. **Sample autoradiogram. Autoradiogram of** a transfer hybridized to pBR322. pBR322 DNA was labelled as described by Feinberg and Vogelstein (1984 Anal. Biochem. 137:266-267). The right half of the figure is a duplicate of the left half except that the spheroplasting step was omitted, with an evident loss of hybridization signal. Three different volumes (0.1, 0.5 or 1.0 ml) of the initial aqueous resuspension were processed and loaded as indicated for each strain examined. These samples were not centrifuged to pellet cellular debris prior to filtration (see step 5). In some samples the greater amount of cellular debris in the larger samples clogs the filter and prevents filtration of the full volume, with the result that hybridization signal is greater in dots to which less material was applied (for examples, see spheroplasted row E, first [0.1 ml] versus second [O.5 ml] and third [1.O ml] dots, and non-spheroplasted row D, first versus second and third dots). This problem can be reduced by pelleting out cellular debris from the alkaline lysate and filtering only the supernatant (see step 5). For more accurate quantitation, we would recommend processing smaller volumes of initial resuspensions (e.g 0.1 ml; see also step 2) and pelleting the cellular debris prior to filtration. However, for the simple determination of presence or absence of pBR322 DNA, this is not necessary. Controls are as follows: untransformed bd; inl; frq^9; a (row A, first three) transformed bd;frq^9; A (row B, first three, untransformed bd; A (row H, second three), transformed bd;inl;frq^9; a::cosR3 (row A, second three). Other samples are progeny from a cross of bd;inl;frq^9; a::cosR3 X bd;fr^9; A. CosR3 is an anonymous cosmid from the ordered Vollmer-Yanofsky library.