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Abstract

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Two similar colony blot protocols have been described for Neurospora crassa (Stohl and Lambowitz, 1983 Anal. Biochem. 134:82-85; Paietta and Marzluf, 1984 Neurospora Newsl. 31:40). Both of these procedures involve the growth of Neurospora colonies on nitrocellulose filters, followed by the digestion of the cell wall. After digestion of the cell wall the filters are treated much as for colony hybridization of bacteria (Grunstein and Hogness, 1975 Proc. Nat. Acad. Sci. USA 72:3961-3965). I have developed a simple colony blot procedure that uses Whatman 541 paper. Since Neurospora colonies can grow through the paper, this eliminates the problem of non-adherence occasionally seen when Neurospora colonies are grown on nitrocellulose membranes. Whatman 541 paper has been used for bacterial colony hybridization (Gergen et al. 1979 Nucleic Acids Res. 7:2115-2136). I have also found that cell wall digestion is unnecessary.

I have used this protocol to screen many strains for the presence of the Neurospora transposon, Tad (Kinsey, 1989 Current Genetics, in press), to test for the presence of unselectable markers in transformed strains, and to follow, in crosses, the segregations of unselectable markers such as in vitro generated deletions of non-coding sequences (Fig. 1A) or mating type (Fig. 1B). A detailed protocol for this colony hybridization procedure follows.

1) Use fresh plates supplemented as necessary and containing 1.5% sorbose, 0.1% glucose and 0.1% fructose.

2) Inoculate plates with fresh conidial suspensions. Grids of 20 colonies per plate work well; however, it is possible, in a field of up to 100 colonies per plate, to pick out a colony that has a single copy of unique DNA not shared with the rest of the field.

3) Allow conidia to germinate and grow for from 6 h to overnight at 34°C. Carefully place a sterile (autoclaved) Whatman 541 filter over the plate. Hold the filter with two sterile forceps and touch it to the center of the plate first. Then use the forceps to press the filter gently onto the agar working out from the center. The paper tends to warp or buckle as you go, but once it is wetted it lies flat on the surface. THE FILTERS SHOULD BE DRY WHEN PLACED ON THE AGAR. Pre-wetting the filters will result in numerous satellite colonies. Mark the filters for orientation and number them with pencil or indelible pen that can survive the hybridization. (Pilot SC-UF pens work well).

4) Return the plates to 34°C until the colonies have grown through the paper and are just starting to conidiate. This typically takes about 48 h.

5) Peel the filter from the plate and place it in a dish of chloroform for 30 min. The lid of a glass petri plate works well for this purpose. Up to 5 filters can be extracted at one time in a petri dish lid. Transfer the filters to a dish of acetone and extract again for 30 min. Air dry filters until acetone odor is gone.

6) Place dry filter in reagent A (0.5 N NaOH, 5X SSC) for 20-30 min. For small lots of filters this is easily done in 3 ml puddles of reagent A on plastic wrap; however, if many filters are to be processed, or if there are too many conidia to wet in this fashion, filters can be treated in 200 ml of reagent A. If filters are treated on saran wrap, add an additional 2 ml of reagent A after about 10 min.

7) Transfer filters to reagent B (0.5 M Tris-HCl, pH 7.5, 10X SSC) for 20 to 30 min. Use either of the above techniques.

8) Drain and air dry on 3MM paper overnight. Baking is not necessary.

HYBRIDIZATION OF NEUROSPORA COLONY BLOTS

1) Pre-wet filter with 5X SSC. Prehybridize at 42°C for 4-6 h in hybridization solution (recipe below). Up to 10 filters can be hybridized in 20 ml of solution. This is a complex hybridization mixture (compliments of the Yanofsky lab), but it works well for this procedure. Although I have not made a thorough systematic study, attempts to simplify it, while retaining successful blots, have been unsuccessful.

2) Add denatured and quick cooled, labeled probe. Fragments isolated from agarose and labeled by the random primer method of Feinberg and Vogelstein (1984 Anal. Biochem. 137:266-267) work well with this procedure. Use 1-2 x 10^6 cpm (Cerenkov) counts for up to 20 ml of hybridization solution.

Figure 1. Use of the colony blot procedure to follow the segregation of single copy DNA. A. Progeny of a cross between a normal sequence strain (J857, lys-1 ure-2; cot-1 a) and strain (J1731) with a 1.5 kb deletion (US-2) in the non-coding DNA upstream of the am gene. The colonies were probed with the 1.5 kb HindIII fragment deleted in strain J1731. A total of 20 colonies were present on the blot. Segregation of the deletion (no hybridization) showed the expected linkage to the ure-2 marker segregating in the cross. B. Segregation of A mating-type in a cross. The probe used was an A-specific 1.2 kb EcoRI/HindIII fragment from the plasmid pMTAG-2 (kindly provided by Dr. L. Glass). Diagnosis of the mating-type was confirmed by conventional analysis. All colonies that hybridized to the A-specific probe crossed only with strains of a mating-type.

3) Incubate overnight (16 h) at 42°C with shaking or rocking.

4) Wash twice at room temperature and twice at 65°C with wash solution (2X SSC, 1% SDS) for 20-30 min each. If background persists, wash at 65°C with 0.5X SSC, 1% SDS.

5) Filters can be stripped by boiling for 30-60 min in stripping solution (0.1X SSC, 1% SDS). I have stripped and successfully reprobed the same filters up to seven times.

Hybridization solution: 40% formamide, 10% dextran sulfate, 4X SSC, 20 mM Tris-HCl pH 7.5, 1X Denhardt's solution, 0.1% SDS, 0.25% non-fat dry milk. This solution can be made in large batches and frozen at -20°C. Just prior to use add boiled (10 min) and fast cooled, sheared salmon sperm DNA to prehybridization mix so that the final concentration of salmon sperm DNA is 100 ug/ml. Recipes for SSC, sheared salmon sperm DNA and Denhardt's solution can be found in Maniatis et al. (1982 Cold Spring Harbor Press). -- Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, KS 66103