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The use of a nonradioactive probe in RFLP analysis of Neurospora crassa DNA

Abstract

Our laboratory is investigating the use of nonradioactive alternatives for the synthesis of DNA probes used in hybridization experiments.

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The use of a nonradioactive probe in RFLP analysis of *Neurospora crassa* DNA.

Our laboratory is investigating the use of nonradioactive alternatives for the synthesis of DNA probes used in hybridization experiments. The use of ^32 P-nucleotides can be expensive because of the rapid decay rate, is inconvenient because of the hazards of handling and disposal and can be time consuming if the specific activity of a probe is too low. Finally, we wanted a method of synthesizing probes that could be adapted to use in teaching laboratories where 32P-labeled materials cannot be conveniently used. We report here our results with the Genius ^TM kit from Boehringer Mannheim Biochemicals.

To test the kit, we chose to prepare a nonradioactive probe as part of an experiment to verify the map position of a cloned gene of *Neurospora crassa* employing the technique of Metzenberg et al. (1984 Neurospora Newsl. 31:35-39) which uses restriction fragment length polymorphisms (RFLPs) as genetic markers. The nonradioactive probe was prepared from DNA obtained from a *Neurospora* cosmid library (Vollmer and Yanofsky 1986 Proc. Natl. Acad. Sci. 83:4869-4873). The cosmid DNA was purified with an alkaline lysis procedure followed by isopycnic centrifugation in CsCl-ethidium bromide gradients and was cleaved with EcoRI to give six linear fragments. The DNA was extracted with phenol/chloroform, precipitated with ethanol, resuspended and labeled as described by the manufacturer. Briefly, the double stranded fragments were denatured at 95°C for 10 min and quickly cooled. The single stranded DNA was then hybridized to a random hexanucleotide mixture followed by incorporation of digoxigenin-labeled dUTP in the presence of all other dNTPs except dTTP. The newly synthesized DNA probe was partially purified by ethanol precipitation in 0.4 M LiCl and was resuspended in 10 mM Tris-HCl and 1 mM EDTA, pH 8.0.

Two strains of *Neurospora crassa*, Mauriceville-1c A (FGSC 4416) and Oak Ridge "multicent-2 a" (FGSC 4488) were grown and the DNA prepared as described (Metzenberg and Baisch 1981 Neurospora Newsl. 28:20-21; Stevens and Metzenberg 1982 Neurospora Newsl. 29:27-28). DNA from each strain was cleaved with BamHI, EcoRI, ApaI and XhoI in separate reactions containing approximately 5 ug of DNA and 50 units of enzyme. The fragments were separated by agarose gel electrophoresis and were depurinated by covering the gel with 0.25 M HCl. After washing the gel in water, the DNA fragments were transferred in 0.4 M NaOH by vacuum to a Nytran^TM (Schleicher & Schuell) nylon membrane. The membrane was washed briefly in 2 X SSC and dried for 2 h at 80°C between two sheets of Whatman 3MM paper. Prehybridization of the membrane, hybridization of the probe to the genomic DNA on the membrane and the subsequent immunological/color detection were performed exactly as described by the maufacturer. We have found Nytran^TM nylon membranes to be satisfactory in the procedure whereas some other membranes are not because of a high color background. The concentration of the labeled probe in the hybridization solution is estimated to be 25-50 ng/ml. The first four lanes of Fig.1 contain Mauriceville-1c A genomic DNA cut with the restriction enzyme indicated. The second four lanes contain Oak Ridge "multicent-2 a" genomic DNA cut with the same four restriction enzymes. We estimate that the lower molecular weight bands contain approximately 100 to 200 pg of *Neurospora* genomic DNA. In our experience, bands can be visualized in two to three days after electrophoresis of the DNA.

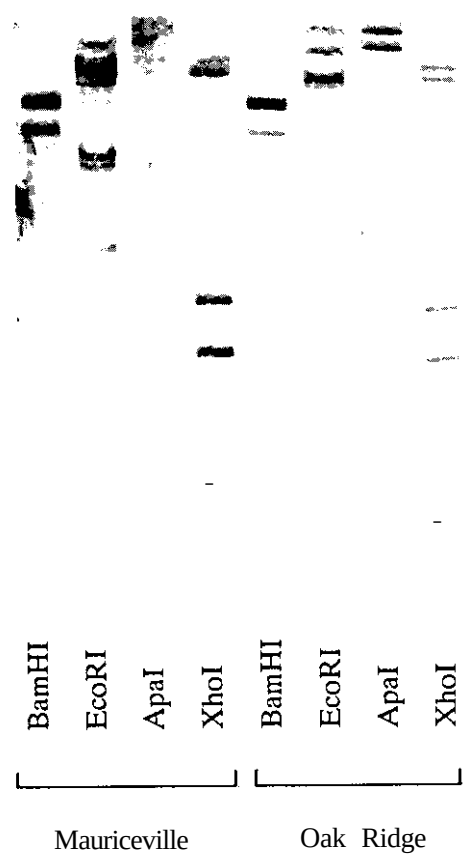

Fig. 1. Hybridization of a nonradioactive probe to *Neurospora* genomic DNA fragments separated on an agarose gel

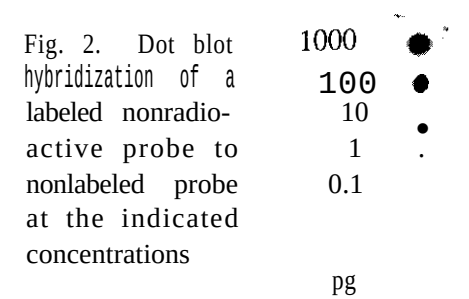

Fig. 2. Dot blot hybridization of a labeled nonradioactive probe to nonlabeled probe at the indicated concentrations

The sensitivity of the method is indicated by the dot blot shown in Fig. 2. A series of spots containing 1000, 100, 10, 1 and 0.1 pg of nonlabeled probe DNA cut with EcoRI was hybridized with the labeled probe at a concentration of 25-50 ng/ml in the hybridization solution. The spot containing 1 pg of nonlabeled probe in easily seen on the membrane. Probes made with the Genius^TM kit have a lifetime of at least one year and the technique is sensitive enough to detect a single gene in 2-5 ug of Neurospora genomic DNA.

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Editors note: We tried this kit with DuPonts GeneScreen and GeneScreen Plus. It does not work with those membranes, at least in our hands.