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

labeled materials cannot be conveniently used. We report here our results with the Genius™ 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 Metzberg 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 (Metzberg and Baisch 1981 Neurospora Newsl. 28:20-21; Stevens and Metzberg 1982 Neurospora Newsl. 29:27-28). DNA from each strain was cleaved with BamHI, EcoRI, ApaI and XhoI in separate reactions containing approximately 5 µg 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™ (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 manufacturer. We have found Nytran™ 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.

Our laboratory is investigating the use of nonradioactive alternatives for the synthesis of DNA probes used in hybridization experiments. The use of <sup>32</sup>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 <sup>32</sup>P-



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

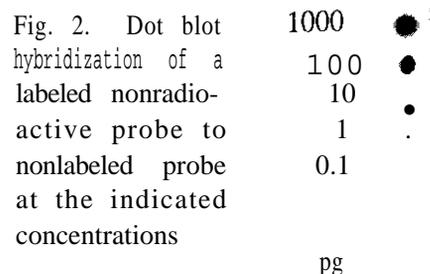


Fig. 2. Dot blot hybridization of a labeled nonradioactive probe to nonlabeled probe at the indicated concentrations

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 is easily seen on the membrane. Probes made with the Genius<sup>TM</sup> 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.