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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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Our laboratory is investigating the use of nonradioactive alternatives for the synthesis of DNA probes used in hybridization experiments. The use of $^{32}\text{P}$-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 $^{32}\text{P}$-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 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 cosmip library (Vollmer and Yanofsky 1986 Proc. Natl. Acad. Sci. 83:4869-4873). The cosmip 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 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 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™ 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.
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™ 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 DuPont's GeneScreen and GeneScreen Plus. It does not work with those membranes, at least in our hands.

Schechtman, M. G.

Segregation patterns of Neurospora chromosome ends: mapping chromosome tips.

I have recently reported the isolation of the chromosomal telomere from linkage group VR (Schechtman, M. 1987. Mol. Cell. Biol. 7:3168-3177). Further work has established that the DNA sequence repeat found at the ends of Neurospora chromosomes is (TTAGGG)n, and that blocks of this repeating sequence are found only at chromosome ends (Schechtman, in preparation). Accordingly, the oligonucleotide (TTAGGG)a has been used as a hybridization probe to determine the segregation pattern for each chromosomal telomere in the genetically marked progeny of the cross multicent-2 a (FGSC 4488) x Mauriceville-1c A (FGSC 2225) (Metzenberg et al. 1984. Neurospora Newsl. 31:35-39; Metzenberg and Grotelueschen 1988. Fungal Genetics Newsl. 35:30-35). Figure 1A shows an autoradiogram displaying a portion of this analysis. Genomic DNA from parental strains and each of the progeny was digested with BamHI, subjected to electrophoresis on an agarose gel, and then to "unblot" hybridization with 32P-(TTAGGG)4 probe (Wallace and Miyada 1987. Meth. Enzymol. 152:432-442). It can be seen that the probe hybridizes to the expected number of bands, fourteen, in each parental lane. Figure 1B is a cartoon representation of the numbered telomere-derived restriction fragments seen in hybridization to the parental DNAs in lanes 1 and 2. Because of the number of bands involved, not all DNA fragments are sufficiently well resolved to permit definitive segregation assignments for each. Only those assignments that can be made with a reasonable degree of certainty are reported here. Table 1 provides the segregation data for seventeen fragments. Four pairs of these fragments are allelic: that is, one band from the Mauriceville parent was found in the progeny as an exclusive alternative of a designated Oak Ridge derived band. Five segregation patterns can be tentatively assigned to particular chromosome ends based on cosegregations with corresponding distal markers. The ends thus mapped are at IIa (R.L Metzenberg, personal communication), IIR, IVR, IIIR, and VR. The end at IVL has also been cloned and shown, by RFLP cosegregation in a separate informative cross, to correspond to the band labelled 010 (Schechtman, Gene, submitted; R.L. Metzenberg and C. Grotelueschen, personal communication). In addition, a list of unassigned segregation patterns is also reported in the table. It should also be noted that in one progeny strain, B7, a new restriction fragment approximately 5.8 kb in length appears, that is present in neither parent. This fragment may have arisen either by reciprocal recombination between two chromosomal tips or by a recombinational repair process such as has been postulated for the spread of repeated subtelomeric X and Y' elements in yeast (Dunn et al. 1984. Cell 39:191-201; Horowitz and Haber 1985. Mol. Cell. Biol. 5:2369-2380).