Agarose Overlays Allow Simplified Staining of Polyacrylamide Gels


Laser scanners from Molecular Dynamics (Sunnyvale, CA, USA) or Hitachi Scientific Instruments (Mountain View, CA, USA) are being used increasingly to detect DNA in polyacrylamide gels. The standard technique for visualizing DNA is to separate the plates of a gel and pour a diluted nucleic acid stain such as ethidium bromide or SYBR® Green I [from Molecular Probes (Eugene, OR, USA); also marketed as Vistra™ Green (Amersham Pharmacia Biotech, Piscataway, NJ, USA)] on the gel. To separate the glass plates, one plate is often treated with an adhesive chemical such as bind silane, and the other plate is treated with a chemical (i.e., Rain-X®) to prevent gel adhesion. Even with the binding treatment, gels have a tendency to float off the plate and wrinkle when the liquid buffer containing the stain is poured on the gel, thus causing difficulty in scoring and a poor image quality. We have found that by mixing the staining buffer with a solid agarose matrix, floating and wrinkling of gels are eliminated. Since they are locked in a solid matrix, the method presented here also greatly facilitates the cleanup of toxic intercalating agents and avoids the problem of accumulating large quantities of liquid hazardous waste. This idea originated from a protocol for staining allozyme gels, in which agar is mixed with the stain and poured on a starch gel to visualize the enzymes (1).

This protocol used sufficient stain to cover a 25- × 32-cm gel. Before pouring the gel, one plate was treated with Rain-X, a consumer glass treatment, to prevent binding of the gel to one plate. No changes in pouring or running of the gels were needed. After electrophoresis, the plates were separated, leaving the gel bound to the plate not treated with Rain-X. The plate with the gel was placed on a level surface. In a 50-mL conical centrifuge tube, 5 µL of Vistra Green nucleic stain were added to 25 mL of double-distilled (dd)H₂O. Twenty-five milliliters of molten 4% agarose (in ddH₂O) were then poured into the tube with the stain solution, giving a final volume of 50 mL with a final concentration of 2% agarose and 0.1 µL/mL of Vistra Green. Final concentrations below 2.0% did not polymerize fast enough to prevent gel wrinkling. After inverting briefly to mix, the stain was poured on the gel and spread evenly across the gel. Some different methods that work for spreading the liquid overlay are a gloved finger, tilting or angling the acrylamide gel, spreading with a pipet tip and spreading with the side of a test tube. Note: such a thin layer of agarose will solidify within 30 s, so one must work quickly. The gel was left to stain in the dark for 15 min. The bottom of the glass plate was cleaned, and the gel was scanned on a Model 595 FluorImager® (Molecular Dynamics). Figure 1 is a comparison of 2% agarose vs. 1% agar. Agar was originally tried because it is less expensive, but results in a darker background than agarose. Figure 2 shows a representative microsatellite gel stained with the overlay procedure.

Figure 1. Background comparison of 2% agarose and 1% agar overlays. Agarose is a 2% solution of SeaKem™ LE Agarose (FMC Bio-Products, Rockland, ME, USA) in ddH₂O, and agar is a 1% solution of Bacto-Agar (Difco Laboratories, Detroit, MI, USA) in ddH₂O. The glass plate with the agarose and agar overlays was scanned using a Model 595 FluorImager at an excitation wavelength of 488 nm and with a 530-nm emission filter at 750 V at normal sensitivity. Relative fluorescence units (rfu) are shown.

Figure 2. Microsatellite locus stained using agarose overlay. Fifteen sturgeon (Scaphirhynchus sp.) individuals were examined for the microsatellite locus Afu-68 on a 5% denaturing polyacrylamide gel. Alleles are designated by numerals with the genotypes noted on the top of the gel. The allelic ladders were made by combining individuals with known genotypes. The sizes of the alleles run from 113 (allele 1) to 145 bp (allele 9). The variation was visualized using a Model 595 FluorImager with settings as described in the Figure 1 legend.

REFERENCE


Address correspondence to Dr. Jeff Rodzen, Dept. of Animal Science, Meyer Hall, University of California, Davis, Davis, CA 95616, USA. Internet: jarodzen@ucdavis.edu

Received 6 April 1998; accepted 22 June 1998.

Jeff A. Rodzen, Jeremy J. Agresti, Gregory Tranah and Bernie May
University of California
Davis, CA, USA