A general and sensitive method for staining DNA and RNA blots

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A method has been developed for the staining of DNA and RNA on nitrocellulose or nylon filters. Histones are bound to the DNA or RNA and stained with india ink (1). Detection of DNA and RNA amounts of less than 10 ng per single band is possible following this simple and inexpensive procedure.

Method: Upon completion of a blot hybridization experiment involving filter-bound nucleic acids, the dry filters are washed twice in 10 mM NaH_2PO_4 pH 7.2, 50 mM NaCl, 0.1% Tween for 10 minutes. Filters are then transferred to 100 ml of the same buffer containing 0.01% (v/v) india ink (Pelikan fountain india drawing ink for fountain pens, Pelikan AG, Hanover, FRG) and 1.0-2.5 $\mu\text{g/ml}$ calf thymus histones (Boehringer, Cat. No. 223 565 or Sigma, Type II-A) and slowly agitated at room temperature for 15-18 h. After staining, the nitrocellulose filters are washed briefly with water and then air dried. Histones were stored as a 5 mg/ml stock solution in 50 mM Tris/HCl pH 7.6 at -80°C . Repeated thawing and freezing led to no appreciable loss in DNA- and RNA-binding.

Comments: The described staining procedure can be used with blots containing DNA of various origins and physical states prior to denaturing (ssDNA, dsDNA, ccc dsDNA). No preferential staining of specific DNA regions has been observed. This method is more sensitive than methylene blue for staining RNA blots. Moreover, it allows DNA markers to be used on RNA blots.

Best results are obtained when Denhardt's solution with or without denatured DNA was present in the hybridization solution prior to staining.

Uses: This staining technique enables easy correlation of hybridization signals with specific bands. Furthermore, transfer errors and transfer efficiency may be checked. The described technique has also been used for inspection of dot blots and for visualization of lysed colonies on colony hybridization filters. Potential applications include hybridization experiments with DNA or RNA probes conjugated to a protein, making hybridization complexes detectable by staining. Furthermore, protein-mediated DNA/RNA staining, using a sequence-specific protein in the presence of india ink, may allow detection of distinct binding sites on DNA or RNA regions.

Reference

1. Hancock, K. and Tsang, V.C.W. (1983) *Anal. Biochem.* **133**, 157-162