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Update on DNA/RNA/Protein Applications and Procedures

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R.H., J. Leavitt, L.E. Hood
H. Kent. 1986. *In* Methods
Sequence Analysis. K.
d.). Humana Press. Clifton,
ey. 277-294.
R.H., J. Leavitt, R.A.
L.E.Hood and S.B.H. Kent.
c. Natl. Acad. Sci. USA.
974.
R.H., G. Pipes, L.E. Hood,
H. Kent. Submitted. 1988.
M.W. & Hood, L.E. 1983.
s in Enzymology. Academic
w York. Vol. 91: 486-494.
. and R.D. Kornberg. 1975.
Acad. Sci. USA.
630.

Western Blotting of Histones to S&S NC™ Nitrocellulose Membranes

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The transfer of histones to nitrocellulose from polyacrylamide gels is often inefficient and highly variable; irreproducible patterns and poor sample binding after blotting make standard solid phase analyses of these structures difficult. We observed that the detergent Triton X-100, commonly included in acetic acid/urea (AUT) gels for the separation of variant forms of the nucleosomal core histones, prevented faithful immobilization of the native histones to NC. We also found that in these gels, and in standard SDS-PAGE gels, the transfer of the highly basic histones was incomplete in standard buffers due to uneven migration out of the gel. Treatment of the AUT gel in an SDS solution removed the Triton without loss of gel resolution. Transfer in an excess of SDS improved blotting from standard SDS-PAGE gels and AUT gels by maintaining sample migration in a uniform direction. We also determined that the retention of histones on the nitrocellulose depended on the presence of methanol in the transfer buffer.

Methods
Histones from calf thymus were separated in acetic acid urea gels as described previously (Bonner, et al. 1980; Mende et al. 1983), and in SDS polyacrylamide gels (Thomas and Kornberg, 1975). After electrophoresis, AUT gels were equilibrated in 50 mM acetic acid containing 0.5% SDS to replace the histone-Triton X-100 complexes in the gel by histone-SDS complexes. Treated AUT gels, and untreated standard SDS gels, were then equilibrated in transfer buffer

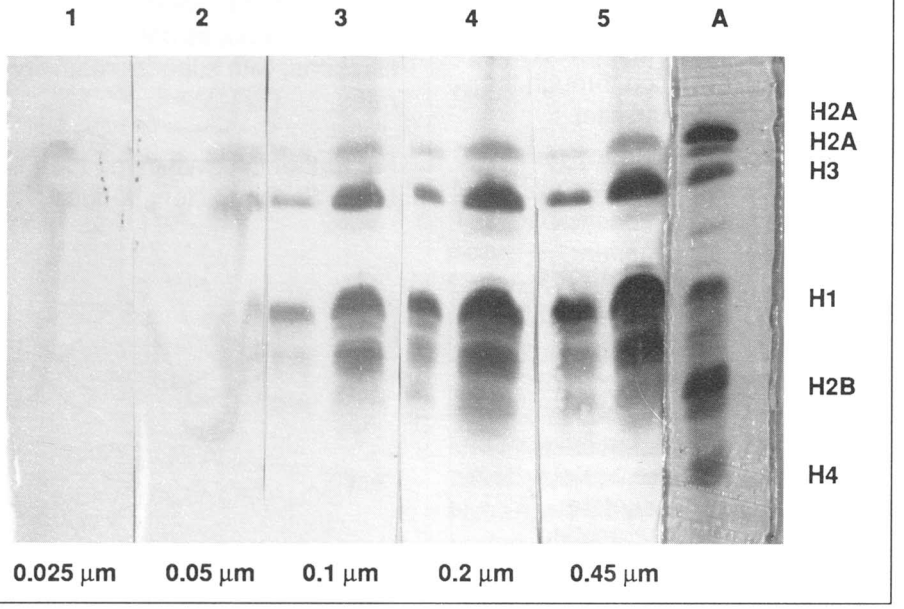
(25 mM Tris/192 mM glycine/20% methanol/0.1% SDS) and blotted for 60 min at 300 mA (50V) at room temperature to S&S NC™ nitrocellulose membranes (0.2 μm). Following blotting, the membranes were washed twice in fresh 10 mM potassium phosphate buffer pH 7.2 with 0.3% Tween 20 and stained with India ink. Gels were stained with Coomassie blue according to standard procedures.

Effect of Gel Constituents on Histone Transfer

The transfer of histones from acetic acid urea gels to nitrocellulose membranes in 0.7% acetic acid becomes erratic and incomplete in the presence of the detergent Triton X-100, which forms complexes with the hydrophobic histones. Free histones readily bind to nitrocellulose membranes, but histone-Triton complexes do not. SDS, urea, and CTAB (cetyltrimethylammonium bromide) are known antagonists to this complex formation. Our studies demonstrated that CTAB, like Triton, inhibited binding of histones to NC, 2-8 M urea did not inhibit retention, nor did SDS in concentrations of 0.5-1.0%. Of these, only SDS, if added in molar excess to histones complexed to Triton, was effective in restoring binding of histones to NC. Further experimentation demonstrated that acidic conditions at low ionic strength facilitated the displacement of Triton by SDS while preventing band diffusion. The marker dye, methylene blue, is also retained in the gel and transferred effectively, allowing easy orientation of the blot with the gel. AUT gels treated in this way behaved identically in subsequent gel equilibration and transfer as standard SDS gels.

Influence of Transfer Buffer Constituents

Omission of SDS from the transfer buffer resulted in increased histone band diffusion and decreased recovery on the nitrocellulose membranes, presumably due to dissociation of the relatively weak complex between SDS and basic protein components. The positively charged, SDS-free histones will migrate in the opposite direction from the negatively charged SDS-histone complexes, resulting in uneven transfer. The addition of excess detergent gave the histone sample a uniform charge and maintained the histone-SDS complex, eliminating transfer variability. Omission of the 20% methanol from the transfer buffer did not affect histone mobilization from the gel or the transfer process, but did affect the retention of the histones on the membrane.



Electrophoretic transfer of calf thymus histones from AUT gels to S&S NC nitrocellulose membranes with different pore sizes. Lane A: 30 μg total calf thymus histones, Coomassie blue stained gel. The major calf thymus histone species are indicated. Lanes 1-5: Histones blotted on S&S NC membranes with pore sizes of 0.025 μm, 0.05 μm, 0.1 μm, 0.2 μm and 0.45 μm.

Effect of NC Properties

Additional studies demonstrated the effect of the properties of the NC membranes on retention efficiency. After extended transfer periods, even in the presence of methanol, histones transferred through the initial membrane (0.2 μm), indicating that the histone-NC complex may not be completely stable. This phenomenon occurred particularly with histone species with a high affinity for Triton, and when the apparent capacity of the NC membrane (approximately 5-30 μg of histone protein per band) was exceeded. The relative retention of histones on NC filters with different pore sizes can be judged from figure below (S&S BA83 0.2 μm membranes are generally recommended).

The treatment of AUT histone gels with SDS in acetic acid prior to transfer of the separated core histone variant proteins to NC membranes allows standard solid phase analysis, similiar to the studies of histone H1 variant proteins separated in SDS gels. The finding that CTAB inhibition of histone-NC binding can be eliminated by SDS, like Triton, may permit blotting of histones from acid/urea/CTAB gels used for second dimensional analysis of histones as well.

For further information on the immobilization of basic proteins on NC, request 350. For information on NA49 CM cation-exchange membranes, request 360, 376, 394.

Bonner, W.M., M.H.P. West and J.D. Stedman. 1980. Eur. J. Biochem. 109:17-23.

Mende, L.M., J.H. Waterborg, R.D. Mueller, and H.R. Matthews. 1983. Biochemistry. 22:38-51.

Thomas, J.O. and R.D. Kornberg. 1975. Proc. Natl. Acad. Sci. USA. 72:2626-2630.

Waterborg, J.H. and R. E. Harrington. 1987. Anal. Biochem. 162:430-434.