A RAPID METHOD TO DETECT DNA BINDING PROTEINS

R. Henschke and E. Nucken Institute of Genetics
University of Bonn, Federal Republic of Germany

Until recently the most useful method of studying DNA binding proteins employed radioactive labelled DNA (1, 2, 3). Working on DNA binding proteins in chloroplasts of Pisum sativum, we have developed a simple method which operates without labelled substances. This test is suitable for detecting the DNA binding ability of diverse protein fractions during the isolation procedure.

Assay procedure. 100 mkl of the DNA-free protein sample (in TEDP-buffer containing 20 mM Tris/HCl pH 7-4, 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, 0.5 mM phenylmethansulfonyl fluoride, 0.05 M NaCl) are incubated with 20 mkl of double stranded calf-thymus DNA (2 mg DNA/ml H_2O) for 10 minutes at 30C. Then 50 mk1 of the mixture are suctioned through a nitrocellulose filter (Schleicher & Schull, BA 85, 0.45 m, pretreated by washing with 3 ml H_2O). In this step proteins bind to the nitrocellulose, whereas free double stranded DNA molecules pass through the filter. If samples contain binding proteins, DNA-protein complexes are formed and so nucleic acid molecules will be retained on the nitrocellulose. To remove unspecifically bound DNA, the filter is washed two times with 3 ml of TEDP-buffer. The filter is dried at 50C for 10 minutes and incubated in ethidium bromide solution (10 mkg 2,7-diamino-10-ethyl-9-phenyl-phenanthridiumbromide/ml) for two minutes. Ethidium bromide intercalates into the DNA, which can be detected by UV-light induced fluorescence. Under these conditions only the spots of those fractions containing DNA binding proteins are visible.

In addition, the following standards are necessary:

a) Positive control. 100 mkl of purified DNA binding proteins (calf histones, 250 Pg/ml, a kind gift from M. Herlt) are incubated with 20 1 of DNA solution (2 mg/ml). 50 pi of this mixture are assayed.

b) Negative control. Corresponding to the positive control, lOOmkl of a non-DNA binding protein (bovine serum albumine, 50 mg/ml) are incubated with DNA.

Furthermore, the sensitivity of the assay was determined by different concentrations of histones. Even 10 mkg DNA binding proteins per 100 mkl were detectable.

The described test is useful to discover DNA binding proteins both from the nucleus and from the organelles.

1. Tsai, R. L. and H. Green. 1973. J. Molecular Biol. 73:307-316.
2. Kohiyama, M., R. Kollek, W. Goebel, and A. Kcpes. 1977. J. Bacterial, 129(2):658-677.
3. Jacq, A. and M. Kohiyama. 1980. European J. Biochem. 105:25-32.