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DNA–protein interactions detected by silver staining

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The mobility shift assay (EMSA) is one of the most powerful methods for the analysis of DNA–protein or RNA–protein complexes. These specific interactions are essential to many basic processes including the control of gene expression, site-specific recombination, replication and the repair of DNA damage. The most important applications of this technique are the kinetic and thermodynamic characterization of complex formation, the definition of binding sites, and conformational analysis.

In order to perform a gel shift assay, the DNA fragments and the proteins (crude extracts or purified) are mixed together. The reaction mixtures are then run on non-denaturing polyacrylamide or agarose gels. Protein binding to the DNA fragments can result in a complex with reduced electrophoretic mobility relative to the free DNA. Various techniques have been reported for detecting DNA–protein complexes. The complexes can be visualized using either ^{32}P- or ^{33}P-dNTP-labelled DNA fragments (Ref. 1, 2) or fluorescently labelled oligonucleotides (Ref. 3). Alternatively, the complexes can be detected by ethidium bromide staining (Ref. 4).

We report here an innovative technique to detect DNA–protein complexes easily in polyacrylamide gels. The technique is performed using purified protein, and complexes are developed by staining the gel with silver nitrate. The main advantages of this technique are

easy manipulation,
the use of reagents that do not require extreme care,
acceptable sensitivity range,
good resolution, and
low cost of reagents and laboratory equipment.

This technique was tested using the main negative regulator that mediates carbon catabolism repression in *Aspergillus nidulans* (CreA) and a DNA fragment containing its binding site (Ref. 5). Other DNA–protein pairs were also tested (data not shown). The recombinant plasmid pET22b::CreA(43–137) contains the CreA zinc-finger domain. The recombinant protein was produced in *Escherichia coli* BL21 and then purified on a $Ni^{2?}$-NTA resin (Qiagen, Valencia, CA, USA). The DNA sequence was a 183 bp *Nhe*I–*Cla*I restriction fragment from a construct that contains the *prnB–prnC* intergenic region of the proline degradation gene cluster (Ref. 6). This fragment was purified from an agarose gel using DEAE cellulose membranes (Schleicher & Schuell NA-45).

1. Protocol

The incubation reactions containing the DNA fragment are carried out at room temperature for 10 min with 1 mg μl^{-1} poly-dIdC, 0.4 mM spermidine, 80 mM KCl in 25 mM Tris-HCl (pH 7.9), 10% glycerol and the recombinant protein. The reactions were then run at 10 V cm^{-1} in a non-denaturing 6% acrylamide–bisacrylamide (30:0.36) gel in 0.5× TBE buffer at 4°C (Ref. 7).

After electrophoresis, the gels were silver stained using the procedure of Merril *et al*. (Ref. 8) modified as follows.

1. Completely immerse the gel in a fixing solution (0.5% glacial acetic acid and 95% ethanol, freshly prepared) and gently agitate for 5 min.
2. Incubate the gel in 0.2% silver nitrate solution and shake for 8 min.
3. Rinse the gel in a minimum volume of distilled water for up to 10 sec.
4. Immerse the gel in an ice-cold (8°C) developer solution (0.75 M sodium hydroxide). Add formaldehyde to a final concentration of 0.66% and agitate gently until optimal image intensity (the best balance between high band intensity and low gel background) is obtained.
5. Immerse the gel in the fixing solution for at least 30 min and then air dry it.

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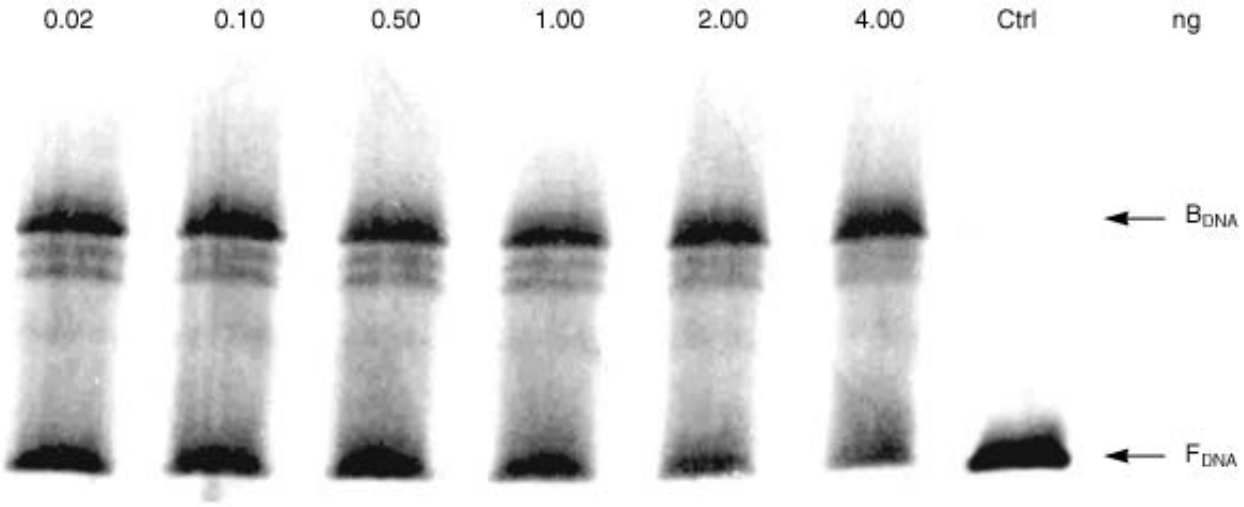

Fig. 1. Electrophoretic mobility shift assays stained with silver nitrate. The lanes were loaded with the indicated amounts of fusion protein, with no protein loaded in the control lane (Ctrl). F_{DNA} and B_{DNA} mark the position of the 183 bp DNA, either free or bound to CreA, respectively.

The protocol described above was performed several times (at least in triplicate).

Figure 1 shows an EMSA developed with silver nitrate. The smallest amount of DNA needed to detect both free and bound DNA was 60 ng lane^{-1} (3.5 pg mm^{-3}). The experiments were performed using increasing amounts of the protein. Figure 2 shows the same experiment as described above but using (in each reaction) 2 ng DNA that had been α-^{32}P-dATP end-labeled by the 'filling in' technique. Experiments using ethidium bromide staining required 600 (ng DNA) lane^{-1} (35 pg mm^{-3}) to distinguish between bound and free DNA (data not shown). This technique shows lower sensitivity than our reported technique.

As expected, the use of radiolabeled probes allow high sensitivity, because the complexes can be detected with only 2 (ng DNA) lane^{-1} (0.1 pg mm^{-3}). Although our method is lower sensitive than the radiolabeling technique, it has the advantages of being practical, easy to perform,

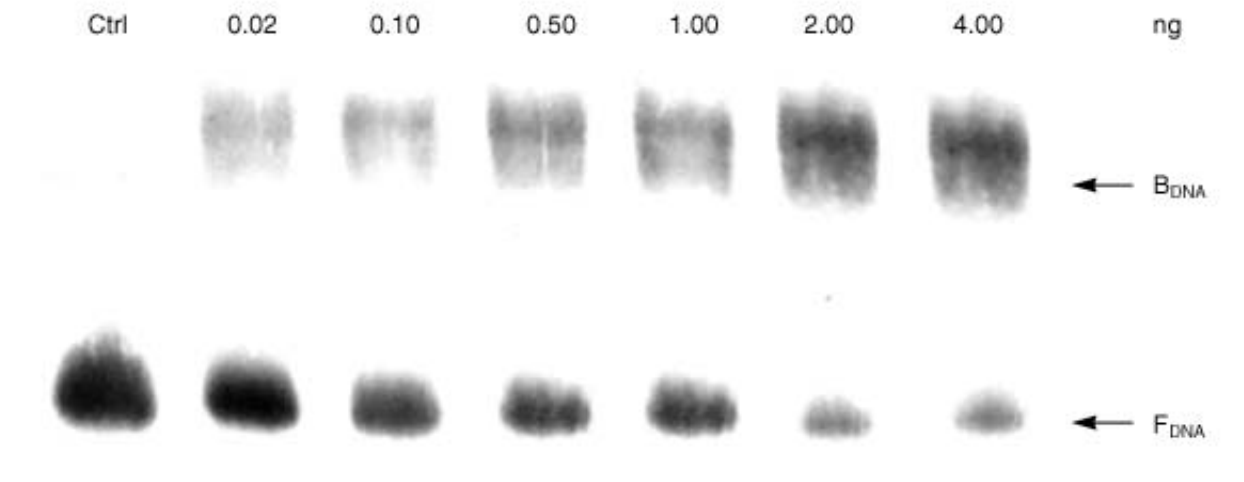

Fig. 2. Autoradiograph of an electrophoretic mobility shift assay. The lanes were loaded with the indicated amounts of fusion protein, with no protein loaded in the control lane (Ctrl). F_{DNA} and B_{DNA} mark the position of the 183 bp DNA, either free or bound to CreA, respectively.

cheap and not requiring either extreme care or special equipment. These factors are always carefully considered when selecting the appropriate detection system. Moreover, our protocol avoids the health risks for workers caused by the reagents involved in the other two techniques.

We conclude that the silver staining technique that we report here might represent an improved and real alternative for performing EMSA with purified protein.

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Products Used

DEAE Cellulose Membranes: DEAE Cellulose Membranes from Schleicher & Schuell