Protocol

Gel Retardation Assays for DNA-binding Proteins

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This protocol was adapted from Molecular Cloning, 3rd edition, by Joseph Sambrook and David W. Russell. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2001

INTRODUCTION

This protocol exploits differences in electrophoretic mobility through a nondenaturing polyacrylamide gel between a rapidly migrating target DNA and a more slowly migrating DNA-protein complex.

MATERIALS

10x Tris-glycine buffer

*TBE buffer may be used in place of Tris-glycine.*

6x Gel-loading buffer I

$^{32}$P-labeled control DNA

$^{32}$P-labeled target DNA of >20 bp (specific activity $\geq 2 \times 10^7$ cpm/µg)

Control nuclear extract or protein fraction

Ficoll 400 (20% w/v)

Dissolve the Ficoll in sterile $H_2O$ and store the solution frozen in 100-µl aliquots at -20°C.

Nuclear extract or Protein fraction(s)

Prepare the extract by one of the methods described in Mapping Protein-binding Sites on DNA by DNase I Footprinting.

Poly(dl-dC) (1 mg/ml)

Labeling of the DNA fragment can be accomplished by phosphorylation (Dephosphorylation of DNA Fragments with Alkaline Phosphatase or Phosphorylating the 5' Termini of Oligonucleotides), filling in of a 3'-recessed end using a DNA polymerase (Labeling 3' Termini of Double-stranded DNA Using the Klenow Fragment of E. coli DNA Polymerase I or Labeling of Synthetic Oligonucleotides Using the Klenow Fragment of E. coli DNA Polymerase I), or by using PCR to incorporate radiolabeled nucleotides into the body of the probe (The Basic Polymerase Chain Reaction).
Dissolve an appropriate amount of poly(dI-dC) in sterile H₂O and store the solution in 100-µl aliquots at -20°C.

Polyvinyl alcohol (10% w/v)

Dissolve the polyvinyl alcohol in sterile H₂O and store the solution frozen in 100-µl aliquots at -20°C.

METHOD

1. To a sterile 1.5-ml microcentrifuge tube add:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>³²P-labeled target DNA</td>
<td>1 ng (1-10 fmoles)</td>
</tr>
<tr>
<td>1 mg/ml poly(dI-dC)</td>
<td>1 µl</td>
</tr>
<tr>
<td>nuclear extract (5-10 µg)</td>
<td>≤10 µl</td>
</tr>
<tr>
<td>or</td>
<td></td>
</tr>
<tr>
<td>protein fraction</td>
<td>≤10 µl</td>
</tr>
<tr>
<td>20% Ficoll 400</td>
<td>5 µl</td>
</tr>
<tr>
<td>or</td>
<td></td>
</tr>
<tr>
<td>10% polyvinyl alcohol</td>
<td>4 µl</td>
</tr>
<tr>
<td>H₂O</td>
<td>to 20 µl</td>
</tr>
</tbody>
</table>

Include control reactions with every experiment. Positive control reactions contain a nuclear extract (or protein fraction) and a radiolabeled DNA fragment carrying a sequence recognized by a DNA-binding protein that is abundant in the extract and has high affinity for the DNA sequence. Examples are a DNA fragment containing an Sp1, C/EBP, or NF-1 site and mammalian cell nuclear extract, or a lacI recognition site and extract derived from a lacI\textsuperscript{q} strain of E. coli. The negative control reactions contain the radiolabeled target DNA fragment, but no nuclear extract.

2. Centrifuge the reaction tubes for several seconds in a microcentrifuge to deposit the reaction mixtures at the bottom of the tubes. Incubate the reactions for 10-30 minutes on ice.

3. Add 3 µl of 6x gel-loading buffer I to each tube. Load the samples into the slots of a neutral 4-7% polyacrylamide gel.

4. Run the gel in either 0.5x Tris-glycine buffer or 0.5x TBE buffer at 200-250 V and 20 mA for ≥2 hours.

Depending on the lability of the binding protein(s) and the affinity of the binding reaction(s), it may be necessary to run the gel at 4°C.

5. After electrophoresis is complete, pry the gel plates apart, transfer the gel to a piece of sturdy blotting paper, and dry the gel for approx. 1 hour on a gel dryer.
6. Expose the dried gel to X-ray film for ≥1 hour at -20°C to visualize radiolabeled DNA fragments. Less abundant DNA-protein complexes can be detected after 1-3-hours on a phosphorimager.

REFERENCES


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**Caution**

**Radioactive substances**

Radioactive substances: When planning an experiment that involves the use of radioactivity, consider the physico-chemical properties of the isotope (half-life, emission type, and energy), the chemical form of the radioactivity, its radioactive concentration (specific activity), total amount, and its chemical concentration. Order and use only as much as needed. Always wear appropriate gloves, lab coat, and safety goggles when handling radioactive material. X rays and gamma rays are electromagnetic waves of very short wavelengths either generated by technical devices or emitted by radioactive materials. They might be emitted isotropically from the source or may be focused into a beam. Their potential dangers depend on the time period of exposure, the intensity experienced, and the wavelengths used. Be aware that appropriate shielding is usually made of lead or other similar material. The thickness of the shielding is determined by the energy(s) of the X rays or gamma rays. Consult the local safety office for further guidance in the appropriate use and disposal of radioactive materials. Always monitor thoroughly after using radioisotopes. A convenient calculator to perform routine radioactivity calculations can be found at: [http://www.graphpad.com/calculators/radcalc.cfm](http://www.graphpad.com/calculators/radcalc.cfm).

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**Recipe**

**6x Gel-loading Buffer I**

- 0.25% (w/v) xylene cyanol FF
- 40% (w/v) sucrose in H₂O
- 0.25% (w/v) bromophenol blue

Store at 4°C.
Recipe

Ficoll 400

To prepare a 20% (w/v) of Ficoll 400, dissolve the Ficoll in sterile H2O and store the solution frozen in 100-µl aliquots at -20°C.

Recipe

Polyvinyl alcohol

Dissolve the polyvinyl alcohol in sterile H2O and store the solution frozen in 100-µl aliquots at -20°C.

Recipe

Tris-glycine buffer

Prepare a 5x stock solution in 1 liter of H2O.

15.1 g Tris base

94 g glycine (electrophoresis grade)

50 ml of 10% SDS (electrophoresis grade)

The 1x working solution is 25 mM Tris-Cl/250 mM glycine/0.1% SDS. Use Tris-glycine buffers for SDS-polyacrylamide gels.