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## Electrophoretic Mobility Shift Assay (EMSA) for Detecting Protein-Nucleic Acid Interactions

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### Abstract

The gel electrophoresis mobility shift assay (EMSA) is used to detect protein complexes with nucleic acids. It is the core technology underlying a wide range of qualitative and quantitative analyses for the characterization of interacting systems. In the classical assay, solutions of protein and nucleic acid are combined and the resulting mixtures are subjected to electrophoresis under native conditions through polyacrylamide or agarose gel. After electrophoresis, the distribution of species containing nucleic acid is determined, usually by autoradiography of <sup>32</sup>P-labeled nucleic acid. In general, protein-nucleic acid complexes migrate more slowly than the corresponding free nucleic acid. In this article, we identify the most important factors that determine the stabilities and electrophoretic mobilities of complexes under assay conditions. A representative protocol is provided and commonly used variants are discussed. Expected outcomes are briefly described. References to extensions of the method and a troubleshooting guide are provided.

### Keywords

Nucleic acid-protein interactions; Gel retardation; Electrophoretic mobility shift assay; Binding detection; Binding analysis

## INTRODUCTION

The electrophoretic mobility shift assay (EMSA) is a rapid and sensitive method to detect protein-nucleic acid interactions<sup>1–6</sup>. It is based on the observation that the electrophoretic mobility of a protein-nucleic acid complex is typically less than that of the free nucleic acid (Fig. 1). The current, widely-used assay differs little from that originally described by Fried and Crothers<sup>7</sup> and Garner and Revzin<sup>8</sup>, although precursors to the technique can be found in the earlier literature<sup>9–11</sup>. Mobility-shift assays are often used for qualitative purposes, although under appropriate conditions they can provide quantitative data for the determination of binding stoichiometries, affinities and kinetics<sup>3, 6, 12</sup>. Methods used in performing the assay differ for each purpose, and a large number of variants have been described in the literature (see Table 1).

### Advantages and limitations of EMSA

The mobility shift assay has a number of strengths. The basic technique is simple to perform, yet it is robust enough to accommodate a wide range of binding conditions (see Table 2 for

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### COMPETING INTEREST STATEMENT

The authors declare that they have no competing financial interests.

representative ranges). Using radioisotope-labeled nucleic acids, the assay is highly sensitive, allowing assays to be performed with small protein and nucleic acid concentrations (0.1 nM or less) and small ( $\leq 20\mu\text{L}$ ) sample volumes. When such high sensitivity is not needed, variants of the assay using fluorescence, chemiluminescence and immunohistochemical detection are also available<sup>13–17</sup>. A wide range of nucleic acid sizes (lengths from short oligonucleotides to several thousand nt/bp<sup>18, 19</sup>) and structures (single-stranded, duplex, triplex<sup>20</sup> and quadruplex<sup>21</sup> nucleic acids as well as small circular DNAs<sup>22</sup>) are compatible with the assay. Under favorable conditions, the distribution of proteins between several nucleic acid molecules can be monitored within a single solution<sup>18, 23</sup>, as can the presence of complexes differing in protein stoichiometry and/or binding site distribution<sup>7, 24</sup>. Proteins ranging in size from small oligopeptides to transcription complexes with  $M_r \geq 10^6$  can give useful mobility shifts<sup>25, 26</sup> and the assay works well with both highly-purified proteins and crude cell extracts<sup>27</sup>. These capabilities account in large part for the continuing popularity of the assay.

On the other hand, the EMSA is not without limitations. One theme of this article is the identification of potential problems and the suggestion of strategies that avoid or mitigate the most severe. Table 3 contains a guide for troubleshooting the most common problems that we have encountered. Perhaps the most important limitation is that samples are not at chemical equilibrium during the electrophoresis step. Rapid dissociation during electrophoresis can prevent detection of complexes, while even slow dissociation can result in underestimation of binding density. On the other hand, many complexes are significantly more stable in the gel than they are in free solution<sup>28–30</sup>; when this is the case, short electrophoresis times allow the resolution of patterns that closely approximate the distributions of species present in the samples at the start of electrophoresis. A second limitation is that the electrophoretic mobility of a protein-nucleic acid complex depends on many factors other than the size of the protein. Thus, an observed mobility shift does not provide a straightforward measure of the molecular weights or identities of proteins that are present in the complex<sup>12</sup>. The electrophoretic “supershift” assay and assays that combine EMSA with western blotting or mass spectroscopy have been devised to allow identification of nucleic acid-associated proteins (summarized in Table 1), while a range of EMSA-based and non-EMSA methods can be used for evaluation of binding stoichiometries<sup>31–34</sup>. A third limitation is that the electrophoretic mobility of a complex provides little direct information about the location of the nucleic acid sequences that are occupied by protein. This information is available from nuclease and chemical footprinting assays that can be performed independently of EMSA or in concert with it<sup>35–38</sup>. Finally, the time resolution of the current assay is defined by the interval required for manual solution handling. This limits kinetics studies to processes with relaxation times significantly larger than the ~1 min required to mix reaction components and for electrophoretic migration into the gel matrix<sup>39</sup>. Strategies designed to improve the time resolution of the technique are in development (M. Fried, unpublished results).

### Alternatives to EMSA

Many techniques are available for the detection and characterization of protein-nucleic acid complexes and most have advantages and disadvantages that differ from those of the EMSA. The most widely used alternative assays are nitrocellulose filter-binding<sup>40, 41</sup> and footprinting<sup>35, 36, 42</sup>.

Filter binding is simple to perform and the manipulations are rapid enough to allow kinetic studies as well as equilibrium measurements<sup>43, 44</sup>. Under favorable conditions, the assay can be highly sensitive and with the exception of the scintillation counter, the required equipment is inexpensive. Like the EMSA, filter binding is a non-equilibrium technique. As a result, quantitative analyses require careful evaluation of filter-retention efficiency<sup>44, 45</sup>. The assay is not limited by the salt concentration of the sample and it can accommodate very large nucleic

acids (e.g., the phage  $\lambda$  genome (48,502 bp<sup>46</sup>)<sup>47</sup>. In contrast, the electrophoretic process limits the salt concentrations of EMSA samples to  $\leq 300$  mM (1:1 salt) and to DNAs of 5000 bp<sup>18, 48, 49</sup> (note that these limits are approximate and may vary with sample and gel compositions). The presence of more than one binding protein complicates filter binding analysis, since retention of labeled nucleic acid is detected, not the identity of bound proteins or the proportion of binding activity attributable to each. In addition, conditions that give efficient filter-retention of 1:1 protein-nucleic acid complexes do not result in significantly increased retention of higher stoichiometry complexes<sup>44</sup>, so the classical assay is poorly suited for distinguishing complexes containing one protein from those containing more than one. Finally, under some solution conditions, single-stranded nucleic acids are retained by nitrocellulose filters<sup>50</sup>, resulting in a background of retained radioactivity that can mask the binding signal. Thus, for applications involving more than one nucleic acid-binding protein, the detection of more than one protein-nucleic acid complex in a mixture and/or binding to single stranded nucleic acids, mobility shift assays offer clear advantages.

Footprinting assays<sup>35, 51</sup> exploit the observation<sup>42</sup> that a protein bound to a specific nucleic acid sequence will interfere with the chemical or enzymatic modification of that sequence. A large number of chemical and enzymatic agents are available for this purpose. In the classical assay, a radioisotope label is located at one end of one strand of the nucleic acid target. Following modification and any steps needed to cleave the nucleic acid at modification sites, fragments are resolved on a denaturing polyacrylamide gel. The resulting ladder of bands is visualized by autoradiography and gaps in the array indicate sites of protection by the protein (s) in the test mixture. Comparison of the protection pattern with sequencing reaction products can allow identification of protected sequences with single-nucleotide resolution. In addition to protection, the appearance of sites that are hypersensitive to modification can provide evidence of conformational change in the target nucleic acid<sup>42</sup>. Because most other methods provide little direct information about the identities of protein binding sites, this method remains the “gold standard” for the identification of nucleic acid sequences within or near binding sites. Since sequences of several hundred residues can be resolved on a typical sequencing gel, the technique is well suited for the analysis of the binding of several protein molecules to a single nucleic acid (a strength that footprinting shares with EMSA). In addition, the footprint signal can be obtained under conditions of binding equilibrium for the protein of interest. This is an important advantage over non-equilibrium assays such as EMSA. Variants of the footprinting assay optimized for quantitative detection of binding have been described<sup>35, 52</sup> and time-resolved methods have been developed that allow the analysis of binding kinetics as well as equilibria<sup>53, 54</sup>.

Footprinting assays require simultaneous optimization of binding by the protein(s) of interest and the nucleic acid modification reaction(s) needed to produce the footprint signal. Thus, they are somewhat more difficult to perform than EMSA or filter binding assays. In addition, because the radioisotope label is distributed over many nucleic acid fragments, the detection of binding by footprinting is less sensitive than the detection of binding using EMSA. In addition, incomplete binding results in a footprint pattern that contains contributions from both free and bound nucleic acids. This makes the protected regions indistinct, because the pattern of free nucleic acid is visible within the protected footprint. A popular solution to this problem is to use EMSA to separate complexes from free nucleic acids after the footprinting modification reaction. Following this step with denaturing gel electrophoresis allows one to independently visualize the fragment patterns of free and bound nucleic acid populations<sup>37, 38</sup>. Finally, some proteins that bind nucleic acids without sequence specificity may not produce distinct footprints. For such systems, EMSA and filter binding assays provide binding signals that are easier to interpret than those available from footprinting.

## Strategic considerations that are relevant to EMSA

No single set of binding and electrophoresis conditions works well for all molecular systems. However, several variables can be optimized for the study of a particular interaction, including design of the nucleic acid target, binding reaction conditions and electrophoresis conditions, as discussed below.

**Selection of nucleic acid target**—Short nucleic acids are easily synthesized and inexpensive to purchase. The small number of non-specific protein binding sites in a small DNA or RNA can be advantageous when the binding protein has low sequence-specificity<sup>12</sup>. In addition, electrophoretic resolution of complexes from free nucleic acid is highest with small nucleic acids and this makes possible short electrophoresis times. On the other hand, all binding sites on a short nucleic acid are close to the molecular ends. This can result in aberrant binding due to structural and electrostatic end-effects<sup>55–57</sup>. Longer templates avoid these limitations but contain more non-specific binding sites, migrate more slowly (requiring longer electrophoresis times) and generally give a smaller mobility-shift on protein binding<sup>6</sup>.

In the classical EMSA assay, the electrophoretic mobility of the nucleic acid is monitored. Nucleic acids can be labeled with radioisotopes<sup>58</sup>, covalent or non-covalent fluorophores<sup>13, 59</sup> or biotin<sup>60, 61</sup>. These labels can be detected by autoradiography, fluorescence imaging, chemiluminescent imaging and/or chromophore deposition, respectively. Alternatively, unlabeled nucleic acid can be used in the binding and electrophoresis steps and detected by post-electrophoretic staining with chromophores or fluorophores that bind nucleic acids<sup>7, 8, 14</sup>. Labeling the nucleic acid at the 5'- or 3'-end with (<sup>32</sup>P)-phosphate is a widely used approach, as it is inexpensive, offers great sensitivity ( $\leq 10^{-18}$  mol of 5' ends can be detected on a routine basis<sup>7</sup>) and does not introduce artificial structures that might influence binding. Thus, when radioisotope use is permissible, we consider <sup>32</sup>P-labeling the method of choice for making nucleic acids detectable in the EMSA assay. When radioisotope use is not possible, covalent labeling of nucleic acid with a fluorophore allows both qualitative and quantitative EMSA applications<sup>62</sup>, although with lower sensitivity than is possible with radioisotope-labeled nucleic acids.

**Binding conditions**—Protein-nucleic acid interactions are sensitive to mono- and divalent salt concentrations<sup>63</sup> and pH<sup>64</sup>. Although a typical Tris-based sample buffer is used in the protocol detailed here, good results have been obtained with many different buffers, including HEPES, MOPS, Bis-Tris, Glycine and Phosphate. We favor buffers that approximate physiological salt concentrations and pH and provide any needed co-factors at appropriate concentrations. However, as long as the conductivity of the sample is not excessive, electrophoresis can be carried out with a wide range of sample compositions. This allows the concentration of binding buffer components to be adjusted to optimize complex formation.

**Additives**—Small neutral solutes such as glycerol or sucrose are often used to stabilize labile proteins. Such solutes can also enhance the stabilities of protein-nucleic acid interactions<sup>65, 66</sup> and can be valuable additions to binding reaction mixtures. They are generally effective in the [solute] < 2M range, but less is known about their effects at higher concentrations, where high viscosity and surface tension complicate solution handling. Addition of modest concentrations ( $\leq 0.1$  mg/mL) of a carrier protein (e.g., BSA) to the binding reaction can minimize non-specific losses of binding proteins during solution handling. Similarly, non-ionic detergents are sometimes helpful in maximizing protein solubility<sup>2</sup>. The useful concentrations of detergents vary with the identities of the detergent and the molecular system under study. Protease, nuclease and phosphatase inhibitors can be useful additives, especially when the protein sample is a partially-fractionated cell or nuclear extract<sup>27</sup>. Mixtures of inhibitors are commercially available and should be used according to the supplier's instructions. Finally,

some systems require specific co-factors for correct function. Examples include cAMP for the *E. coli* CAP protein<sup>67</sup>, ATP for recombinases such as *E. coli* RecA or human Rad51<sup>68, 69</sup> and polyamines for some eukaryotic transcription factors<sup>70</sup>. Where necessary, small-molecule additives that stabilize complexes can be included in binding and gel buffers, to stabilize complexes during electrophoresis<sup>3,7</sup>.

**Competing nucleic acid**—Often a protein sample will contain more than one nucleic acid binding activity. When secondary binding activities obscure the one of interest, the addition of unlabeled competing nucleic acid to the reaction mixture can reduce the binding of secondary proteins to the labeled target<sup>27</sup>. This strategy works when the protein of interest binds the target nucleic acid with greater affinity than it binds the competitor and when the secondary binding activities do not discriminate between competitor and target sequences. Since competing nucleic acids also reduce the amount of specific binding, even under favorable conditions, it is best to test a range of competitor concentrations to optimize discrimination of specific and non-specific binding. Commonly used competitors include genomic DNAs, poly d(A–T) and poly d(I–C)<sup>5</sup>.

**Electrophoresis conditions**—The resolution of complexes depends on their stability during electrophoresis. Since many buffers are compatible with electrophoresis, the composition and concentration of gel and running buffer components can be adjusted to optimize the stability of complexes. The most popular buffers are variants of Tris-borate-EDTA<sup>71,72</sup>, Tris-acetate-EDTA<sup>73</sup> or Tris-glycine<sup>27</sup>. As mentioned above, low molecular weight co-factors and/or non-specific stabilizers such as glycerol or ethylene glycol can be included in gel- and running buffers to enhance the stability of complexes. In some circumstances it may be feasible to perform electrophoresis in the buffer that was used in the binding reaction<sup>3, 6</sup>. This avoids subjecting the sample to a change of buffer conditions during gel loading and as reaction components migrate into the gel. This has the advantage of avoiding any perturbation to the molecular system that might be due to a change in buffer composition; it also eliminates the need to independently optimize binding and electrophoresis buffer conditions.

Although both polyacrylamide and agarose gels have been used for EMSA<sup>7,8,74</sup>, polyacrylamide gels offer better electrophoretic resolution for protein-DNA and protein-RNA complexes of  $M_r \leq 500,000$ <sup>3</sup>. In addition, some complexes are significantly more stable in polyacrylamide matrices than in agarose gels or free solution<sup>7,29</sup>. While the mechanism of this effect is not completely understood, for a few molecular systems it has been shown that the dissociation rates of complexes decrease with increasing gel concentration<sup>28, 30</sup>. On the other hand, the resolution of large complexes and the duration of electrophoresis is much longer in high percentage gels. Thus, optimization of gel concentration can be a valuable precursor to the most critical EMSA applications. One effective optimization strategy is to start with a relatively low concentration gel (e.g., 5% w/v acrylamide) and to increase this concentration systematically until any improvement in complex stability is balanced by loss of electrophoretic resolution and/or the onset of impractically long electrophoresis times.

Given below is a protocol for a representative mobility shift assay. The protein is purified *E. coli* cyclic AMP receptor protein (CAP)<sup>49, 67</sup> and the nucleic acid is a 214 bp restriction fragment from the *E. coli lac* promoter-operator region<sup>48</sup>. The binding buffer consists of 10 mM Tris (pH 7.5 at 20°C), 100 mM KCl, 1 mM EDTA, 0.1 mM DTT, 20 μM cyclic AMP, 5% v/v glycerol, 0.010 mg/mL BSA and the electrophoresis was carried out in a 10% w/v polyacrylamide (75:1 acrylamide:bisacrylamide) gel, cast in 40 mM Tris-acetate, 2.5 mM EDTA (pH 7.8 at 20°C) and run in 40 mM Tris-acetate, 2.5 mM EDTA (pH 7.8 at 20°C), supplemented with 20 μM cyclic AMP. The results of this assay are shown in Fig. 1.

## MATERIALS

Some materials and solutions described below are necessary only if the investigator intends to cast his/her own polyacrylamide gels. These are indicated with an asterisk (\*). Pre-cast gels that are available commercially are a convenient and viable alternative to ones made in the laboratory although their use reduces the investigator's ability to modify gel concentration, crosslinking or electrophoresis buffer compositions.

## REAGENTS

- TEMED\* (*N,N,N',N'*-Tetramethylethylenediamine) [Sigma, catalog number T9281]. Store tightly sealed at 4°C.
- Ammonium persulfate\* [Sigma, catalog number A3678]. Store in a desiccator at 4°C.
- Acrylamide monomer\*, electrophoresis grade [Sigma, catalog number A3553]. Store refrigerated at 4°C. Protect from light.
- *N,N'*-methylene bisacrylamide\* [USB Corporation, catalog number 75821]. Store refrigerated at 4°C. Protect from light.
- Other reagents should be "molecular biology grade" or better.

## SOLUTIONS AND PREPARATIONS

- Protein solution or cell extract containing nucleic acid binding activity. Purification methods vary according to the protein in question. Methods for preparing cell- and nuclear extracts that can be used for EMSA have been described elsewhere<sup>75, 76,74</sup>.
  - ▲ **CRITICAL STEP:** The preparation of protein solutions for assay can be time consuming. Since nucleic acid binding activities are often labile, samples should be prepared and stored under conditions that maximize the lifetime(s) of the active protein(s) and minimize the potential for undesirable modifications and proteolysis.
- <sup>32</sup>P-labeled target nucleic acid. Methods are available for labeling DNA and RNA with <sup>32</sup>P at 5' ends, 3' ends, or internally<sup>72,77</sup>.

**! CAUTION** Exposure to beta radiation and secondary X-radiation from <sup>32</sup>P is hazardous. Most research institutions specify procedures for the safe handling of this isotope, which should be followed stringently.

- 10X Tris-acetate EDTA (TAE) electrophoresis buffer. 400 mM Tris, 25 mM EDTA, brought to pH 7.8 with acetic acid. As described in the Introduction, many variations of electrophoresis gel and running buffers can be used. Note: if a commercially prepared gel is used, it is best to use an electrophoresis buffer that matches the one with which the gel was cast. Consult the product literature for information on the selection and preparation of electrophoresis buffers.
- 40% w/v acrylamide-bisacrylamide stock solution\*. Acrylamide monomer, 39.5 g/100 mL; *N,N'*-methylene bisacrylamide 0.5 g/100 mL. Filter the resulting solution through a 0.45 micron filter. Store at 4°C in a brown glass bottle.

**! CAUTION** Acrylamide and bisacrylamide are neurotoxic. Weigh these reagents in a draft-free area. Wear a particle mask, gloves and eye protection when handling acrylamide powders. Never pipet solutions by mouth.

**ALTERNATIVE** Acrylamide-bisacrylamide stock solutions\* can be purchased from commercial sources (for example, 40% w/v acrylamide-bisacrylamide solution for electrophoresis, 37.5:1 acrylamide:bisacrylamide [Sigma, catalog number A7168]).

Although this product is convenient, the higher acrylamide:bisacrylamide ratio (approximately 75:1 w/w) of our formulation results in a gel with fewer crosslinks at a given acrylamide concentration than the 37.5:1 mixture. We believe that the 75:1 formulation gives better resolution of large complexes than do solutions containing greater proportions of bisacrylamide.

- 10X Dye + glycerol stock solution: 10mM Tris, 1mM EDTA, 50 % v/v glycerol, 0.001% w/v bromophenol blue, 0.001% w/v xylene cyanol FF.
- 10X Binding buffer: 100 mM Tris (pH 7.5 at 20°C), 10 mM EDTA, 1 M KCl, 1 mM DTT, 50% v/v glycerol, 0.10 mg/mL BSA. As described in the Introduction, many variations can be used.

## REAGENT SETUP

### Degassing gel polymerization solutions

The composition of the gel polymerization solution is given in Table 4. We have found that polymerization is more efficient and resulting gels more homogeneous if the solution is thoroughly degassed before the polymerization reaction is started. Our equipment for degassing gel solutions consists of a thick-walled side-arm flask with a volume at least 4× greater than the solution to be treated, a stopper for the flask, a magnetic stir bar and stir plate to agitate the solution and a vacuum hose connecting the side-arm flask to a mild vacuum source (“house” vacuum or a water aspirator are sufficient).

**! CAUTION** Always interpose a trap between the side-arm flask and the vacuum source, to prevent contamination of the vacuum source with acrylamide.

The gel solution (*minus the TEMED*) is stirred at room temperature, under vacuum, until bubble formation stops. This typically requires 5–15 min. Polymerization should be started (by addition of *TEMED*) within 30 min of degassing treatment; if this is not possible, degas the solution (*minus the TEMED*) again before use.

**! CAUTION** Always wear eye protection, especially when working with glassware under vacuum.

## EQUIPMENT

- Electrophoresis power supply (250 V, 200 mA capacity recommended, 100 V, 25 mA capacity minimal). Most commercial power supplies sold for SDS-PAGE or DNA sequencing applications are suitable.
- Vertical electrophoresis apparatus. Most commercially available vertical gel electrophoresis systems are suitable. This equipment includes the glass plates, spacers, well-forming combs, clamps and optionally, a gel-casting stand, needed to prepare and to run a polyacrylamide gel. While more elaborate equipment is available, the basic Studier “gel box”<sup>78</sup> works well for most EMSA applications.
- Side-arm flask & stopper, access to mild vacuum or water aspirator, to degas gel mixtures before polymerization.
- Autoradiography film cassette and Kodak XAR-5 film or cassette and storage phosphor screen. Access to a darkroom equipped with film developer is needed for film autoradiography. Access to a phosphorimager instrument is needed for storage phosphor screen autoradiography.
- Plastic food wrap. Types with relatively low “cling” such as Saran Wrap® are easier to work with than others.

- Accurately calibrated air-displacement pipettors (e.g., Gilson Pipetman). Instruments that deliver 1 $\mu$ L–10 $\mu$ L, 2 $\mu$ L–20 $\mu$ L and 20 $\mu$ L–200 $\mu$ L are needed to span the volume ranges given in the example protocol. Standard-taper tips are suitable for most solution handling, but narrow-taper gel-loading tips (e.g., Corning, catalog number 4853) are necessary for the gel-loading step.

## PROCEDURE

### Gel preparation (TIMING <3h)

If commercially available pre-cast gels are used, skip to step 4 (pre-electrophoresis).

1. Ensure that gel plates, spacers and comb are clean. Remove any fingerprints or other residue from glass plates with a swab saturated with methanol. Ensure that all components are dry before continuing.
2. Prepare the polymerization mixture. The amounts of reagents required for one gel with dimensions 0.08  $\times$  12  $\times$  18 cm (nominal volume 17.8 mL), containing 5%–15% w/v polyacrylamide, are shown in Table 4.

**!CAUTION** Acrylamide and bisacrylamide are neurotoxins. Wear gloves and use eye protection when handling the polymerization solution. Never pipet solutions by mouth.

**▲CRITICAL STEP** Polymerization of thoroughly degassed solutions can be rapid. Viscosity can become too great to allow the solution to be poured or transferred by pipet within ~5 min. Therefore, do not start the polymerization reaction until ready to cast the gel.

3. Pour the polymerizing mixture into the glass plate assembly, avoiding bubble formation. Insert the well-forming comb immediately and allow the gel to polymerize for at least 2h. Alternatively, precast native gels can be purchased from vendors, and the protocol can be started from step 4.

**▲CRITICAL STEP** Prevent bubble formation. Since bubbles do not conduct electrical current, they disrupt electrophoretic migration. Thus, it is important to minimize the number and size of bubbles trapped during polymerization. Pour the polymerizing mixture slowly to avoid bubble formation. Tilting the gel-plate assembly 45° from the vertical can minimize bubble formation during pouring. If bubbles are trapped in the solution, finish pouring, stand the assembly on end so that the plates are vertical and gently tap the assembly with a spatula to dislodge the bubbles. Once bubbles have floated to the top of the solution, they should be popped or removed before inserting the well-forming comb.

**•PAUSE POINT** Polymerized gels can be stored at room temperature, with open edges sealed with plastic film, for several hours or up to 1 week at 4°C and 100% humidity. We use a plastic box in a refrigerator for this purpose. A paper wick attached to the side of the box and saturated with water is sufficient to maintain the humidity.

### Pre-electrophoresis (TIMING 0.5–1.0h)

4. Remove comb and bottom spacer (if used) from gel and mount the gel in the vertical electrophoresis apparatus.
5. Fill the upper and lower buffer reservoirs with electrophoresis running buffer. For gels obtained with the polymerization protocol given above, the appropriate solution is 1X concentrated TAE buffer. Use a Pasteur pipette to rinse the submerged surfaces of the sample wells with buffer, to ensure that no debris is present. A Pasteur pipette with a 90°



bend in the narrow end can be useful for displacing air bubbles trapped at the bottom of the gel.

▲**CRITICAL STEP** Charged reagents that are incompatible with the gel polymerization reaction can be moved into the gel by electrophoresis. An appropriate concentration of the reagent of interest is mixed with the running buffer prior to pre-electrophoresis. An example of this is the inclusion of cAMP in the electrophoresis buffer of the assay shown in Fig. 1.

6. Place ~5  $\mu\text{L}$  of 1X dyes + glycerol solution in each well of the gel and conduct pre-electrophoresis at ~10 V/cm of gel length. Reduce this voltage if gel heating is evident.

▲**CRITICAL STEP** The dye migration pattern tests whether the gel density and conductivity are homogeneous and reveals any defect in the shape of the sample well. Discard any gel that does not give narrow, well-resolved dye bands at this stage.

### Sample preparation and equilibration (TIMING 1–1.5h)

7. Prepare samples and equilibrate for 30 min at  $20 \pm 1$  °C. This can be carried out, in part, while the gel is undergoing pre-electrophoresis. A titration protocol is normally used to determine the optimal concentration of protein required to interact with a constant amount of nucleic acid. Sample variation is minimized by preparing a premix of components that are present in constant concentrations and distributing a constant volume of this solution to individual sample tubes. All non-protein components should be mixed and brought to the reaction temperature prior to addition of the protein. This ensures that the protein encounters all solution components at close to their final concentrations. As an example, a typical titration protocol used for the study of the CAP-DNA interaction is given in Table 5. Note that cAMP is a special additive needed for sequence-specific DNA-binding activity in this molecular system.

▲**CRITICAL STEP** Equilibration time will depend on the identities of the interacting molecules and on many of the reaction conditions (examples include but are not limited to temperature, protein and nucleic acid concentrations and salt concentration). Failure to attain equilibrium may give irreproducible results. A minimal control for the attainment of equilibrium is to divide each sample in an assay into two aliquots and to incubate one subset for 30 min and the other subset for 60 min (or more). Analyze the mixtures using identical electrophoresis conditions. Equal mole-fractions of bound and free nucleic acids in corresponding samples are evidence of close approach to binding equilibrium.

### Electrophoresis (TIMING 30 min-5h depending on the electrophoretic resolution required)

8. At the end of the equilibration period, rinse out the wells of the gel and load the samples. In the experiment shown in Fig. 1 (reaction compositions given in Table 5), the entire sample (30  $\mu\text{L}$ ) was loaded. However, smaller sample volumes can be used (a practical lower limit is imposed by the need to cover the bottom surface of the well with a uniform layer of sample solution; for typical gels this can be as small as 5  $\mu\text{L}$ ). Small sample volumes are advantageous because they result in narrow starting zones at the start of electrophoresis and, accordingly, sharper bands at the end of electrophoresis.

Gel loading is conveniently done using an air-displacement pipettor equipped with narrow-taper “gel-loading” tips (see equipment list, above). Many sample compositions are dense enough to layer cleanly at the bottom of the sample wells. For those that are not, inclusion of glycerol to a final concentration of 2–5% v/v will facilitate loading. (Many other dense solutes work equally well for this purpose.) Place the loaded tip within 1 mm of the gel surface and slowly expel the sample. Avoid expelling an air bubble after the sample, since the turbulence this causes will disrupt the narrow zone of the undisturbed sample.

9. Load tracking dye in flanking, unused wells as a marker, and perform electrophoresis at 10 V/cm. Reduce the voltage if the gel becomes warm during electrophoresis. To minimize complex dissociation, the electrophoresis interval should be as short as possible, consistent with resolution of the species of interest. Note that some molecular systems are not perturbed by the addition of tracking dyes to the samples. For these systems it is convenient to add dyes directly to the samples, prior to loading the gel.

### Detection of electrophoretic bands (TIMING 30 min to several days, depending on the radioactivity of the sample)

10. At the end of electrophoresis, remove the gel and plate assembly from the electrophoresis device and dry it thoroughly with paper towels.

▲**CRITICAL STEP** The gel and plate assembly must be dry before separating the plates. Residual buffer trapped between the plates can promote vacuum formation as the plates are pried apart and this can distort the gel. In addition, transfer of free buffer to the gel surface can result in elution of radioactive nucleic acid from the gel during subsequent steps. This “free” radioisotope is a nuisance to autoradiography and a potential safety hazard.

11. Gently separate the plates, leaving the gel adherent to one of the plates. Wrap the gel and plate in a single layer of plastic food wrap. The wrapped surface of the gel should be free of air bubbles and wrinkles, since these will interfere with autoradiography. For short ( $\leq 24$ h) autoradiographic exposures, this preparation is sufficient. For longer exposures, drying the gel on a paper or membrane support or freezing it at  $-80^{\circ}\text{C}$  (see below) will minimize band broadening due to diffusion.

12. Autoradiography. Working in a suitable darkroom (for film autoradiography) or in a laboratory with dim light (for phosphor screen autoradiography) place film or phosphor screen in an exposure cassette. Place the wrapped gel and plate assembly in the cassette, with gel-side toward film or screen. Close the cassette. Expose film or screen at  $4^{\circ}\text{C}$  for intervals up to 24h, and at  $-80^{\circ}\text{C}$  for longer intervals. Note: Flexible cardboard or plastic film cassettes accommodate glass gel plates better than the traditional rigid metal cassette, but they do not absorb beta radiation emitted by the gel. If cassettes are stacked during the exposure interval, separating them with plexiglass sheets (5mm thick) will minimize cross-exposure fogging of adjacent films or phosphor screens. At the end of the exposure period, develop the latent image on phosphor screen or film, according to manufacturer's instructions. Example gel pictures are shown in Figures 1–4.

## TROUBLESHOOTING

The “pilot conditions” described above work well for many binding proteins. However, if binding is not detected the protocol may require modification. Outlined in Table 3 are some common problems, interpretations and solutions.

### TIMING

**Steps 1–3**, Gel preparation: less than 3 hours

**Steps 4–6**, Pre-electrophoresis: 30 min. to 1 hour

**Step 7**, Sample preparation and equilibration: 1 to 1.5h. This step can be initiated during pre-electrophoresis (steps 4–6).

**Step 8**, Electrophoresis: 30min to 5 hours. The time required will vary depending on the experiment.

**Steps 9–12**, Detection of electrophoretic bands: typically 30 min. to several days. Autoradiographic exposure time will depend on concentration of nucleic acid and its specific radioactivity.

## ANTICIPATED RESULTS

In a typical experiment, protein complexes migrate more slowly than free nucleic acid (Fig. 1). The mobility decrement that is observed depends on many factors including the sizes of protein and nucleic acid, the number of protein molecules bound, protein charge, and for rod-like DNAs, whether the DNA is bent on complex formation<sup>3, 79</sup>. Current versions of the EMSA provide little direct information about the location(s) of the binding site(s) that are occupied in protein-nucleic acid complexes. However, footprinting assays<sup>36, 42, 80</sup> carried out in parallel with EMSA provide an efficient means of identifying occupied sequences.

Although the CAP-DNA complex shown in Fig. 1 has a 1:1 stoichiometry, in other systems the first complex to form may have a different protein:nucleic acid ratio. For example, the first detectable complex to form in the titration of a 16 nucleotide (nt) single-stranded DNA with the human AGT DNA-repair protein has a 4:1 stoichiometry<sup>33</sup> (Fig. 2). A range of EMSA methods are available for the determination of stoichiometries<sup>3, 6, 12</sup> and investigators are encouraged to consider non-EMSA methods such as analytical ultracentrifugation<sup>81–83</sup>, as well.

Occasionally, several complexes of differing stoichiometry will form in a single binding reaction. Under favorable conditions, these can be resolved, resulting in a “ladder” of bands in which each stoichiometric step is represented (Fig. 3). When more than one binding protein is present in the reaction mixture, complexes may form containing individual proteins or any possible combination of proteins. An example of such an assay with purified *E. coli* CAP and *lac* repressor proteins is shown in Fig. 4. Partition analysis methods<sup>7, 12, 18</sup> are available for the characterization of multiple equilibria of the kinds shown in Figs. 3 and 4.

When the source of binding activity is an unpurified or partially purified cell extract, the identification of the protein(s) present in a resolved complex is often the most important challenge. Because cell extracts contain large numbers of nucleic acid-binding proteins, it is risky to assume that the dominant binding activity associated with a nucleic acid is the one of interest to the investigator. The classical approach to this problem has been the electrophoretic supershift assay, in which an antibody against a candidate protein reduces the gel-mobility of the protein-nucleic acid complex, producing a secondary mobility shift<sup>84</sup>. More recently, 2-dimensional strategies in which EMSA is paired with SDS-PAGE with Western blot detection<sup>85, 86</sup>, or paired with mass spectrometry<sup>87, 88</sup> have started to bring the power of proteomics to bear on this problem. A detailed protocol describing EMSA paired with SDS-PAGE and mass spectrometry for protein identification has recently been published<sup>89</sup>.

## Acknowledgments

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## ABBREVIATIONS

**EMSA**  
electrophoretic mobility shift assay

**Tris**

	Tris(hydroxymethyl)aminomethane
<b>HEPES</b>	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
<b>MOPS</b>	3-(N-morpholino)propanesulfonic acid
<b>Bis-Tris</b>	1,3-bis(tris(hydroxymethyl)methylamino)propane
<b>BSA</b>	bovine serum albumin
<b>cAMP or cyclic AMP</b>	3'-5'-cyclic adenosine monophosphate
<b>CAP</b>	<i>E. coli</i> cAMP receptor protein
<b>ATP</b>	adenosine triphosphate
<b>EDTA</b>	ethylenediamine tetraacetic acid
<b>TEMED</b>	N,N,N',N'-tetramethylethylenediamine
<b>DTT</b>	dithiothreitol
<b>SDS-PAGE</b>	denaturing polyacrylamide gel electrophoresis carried out in the presence of sodium dodecylsulfate

## References

1. Garner MM, Revzin A. The use of gel electrophoresis to detect and study nucleic acid-protein interactions. *Trends Biol Sci* 1986;11:395–396.
2. Buratowski, S.; Chodosh, LA. *Current Protocols in Molecular Biology*. Ausubel, FM.; Brent, R.; Kingston, RE.; Moore, DD.; Seidman, JG.; Smith, JA.; Struhl, K., editors. John Wiley and Sons; New York: 1996. p. 12.2.11-12.2.10.
3. Fried MG. Measurement of protein-DNA interaction parameters by electrophoresis mobility shift assay. *Electrophoresis* 1989;10:366–376. [PubMed: 2670548]
4. Carey J. Gel retardation. *Methods Enzymol* 1991;208:103–117. [PubMed: 1779832]
5. Lane D, et al. Use of gel retardation to analyze protein-nucleic acid interactions. *Microbiol Rev* 1992;56:509–528. [PubMed: 1480106]
6. Fried, MG.; Garner, MM. *Molecular Biology Methods and Applications*. Tietz, D., editor. Elsevier; New York: 1998. p. 239-271.
7. Fried MG, Crothers DM. Equilibria and Kinetics of Lac Repressor-Operator Interactions by Polyacrylamide Gel Electrophoresis. *Nucl Acids Res* 1981;9:6505–6525. [PubMed: 6275366]
8. Garner MM, Revzin A. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions: application to components of the *Escherichia coli* lactose operon system. *Nucl Acids Res* 1981;9:3047–3060. [PubMed: 6269071]
9. Eisinger J. Visible gel electrophoresis and the determination of association constants. *Biochem Biophys Res Comm* 1971;44:1135–1142. [PubMed: 4946185]

10. Chelm BK, Geiduschek EP. Gel electrophoretic separation of transcription complexes: an assay for RNA polymerase selectivity and a method for promoter mapping. *Nucl Acids Res* 1979;7:1851–1867. [PubMed: 537912]
11. Varshavsky A, et al. Heterogeneity of chromatin subunits *in vitro* and location of histone H1. *Nucl Acids Res* 1976;3:477–492. [PubMed: 1257057]
12. Adams, C.; Fried, MG. Protein Interactions: Biophysical Approaches For The Study of Multicomponent Systems. Schuck, P., editor. Academic Press; New York: 2007. p. 417-446.
13. Rye HS, et al. Stable fluorescent dye-DNA complexes in high sensitivity detection of protein-DNA interactions. Application to heat shock transcription factor. *J Biol Chem* 1993;268:25229–25238. [PubMed: 8227088]
14. Jing D, et al. A sensitive two-color electrophoretic mobility shift assay for detecting both nucleic acids and protein in gels. *Proteomics* 2003;3:1172–1180. [PubMed: 12872218]
15. Mukhopadhyay J, et al. Fluorescence resonance energy transfer (FRET) in analysis of transcription-complex structure and function. *Methods Enzymol* 2003;371:144–159. [PubMed: 14712697]
16. Berger R, et al. Nonradioactive gel mobility shift assay using chemiluminescent detection. *Biotechniques* 1993;15:650–652. [PubMed: 8251167]
17. Rodgers JT, et al. Use of biotin-labeled nucleic acids for protein purification and agarose-based chemiluminescent electromobility shift assays. *Anal Biochem* 2000;277:254–259. [PubMed: 10625515]
18. Fried MG, Daugherty MA. Electrophoretic analysis of multiple protein-DNA interactions. *Electrophoresis* 1998;19:1247–1253. [PubMed: 9694259]
19. Rasimas JJ, et al. Interactions Of Human O6-Alkylguanine-DNA Alkyltransferase (AGT) With Short Single-Stranded DNAs. *J Biol Chem* 2007;282:3357–3366. [PubMed: 17138560]
20. Musso M, et al. The yeast CDP1 gene encodes a triple-helical DNA-binding protein. *Nucleic Acids Res* 2000;28:4090–4096. [PubMed: 11058104]
21. Tolstonog GV, et al. Interaction *in vitro* of type III intermediate filament proteins with higher order structures of single-stranded DNA, particularly with G-quadruplex DNA. *DNA Cell Biol* 2005;24:85–110. [PubMed: 15699629]
22. Nordheim A, Meese K. Topoisomer gel retardation: detection of anti-Z-DNA antibodies bound to Z-DNA within supercoiled DNA minicircles. *Nucleic Acids Res* 1988;16:21–37. [PubMed: 3340525]
23. Surgurladze N, et al. Interactions and reactions of ferritin with DNA. *J Biol Chem* 2004;279:14694–14702. [PubMed: 14734543]
24. Hudson JM, Fried MG. Co-operative interactions between the catabolite gene activator protein and the *lac* repressor at the lactose promoter. *J Mol Biol* 1990;214:381–396. [PubMed: 2166165]
25. Talanian RV, et al. Sequence-specific DNA binding by a short peptide dimer. *Science* 1990;249:769–771. [PubMed: 2389142]
26. Mita BC, et al. Interference of PR-bound RNA polymerase with open complex formation at PRM is relieved by a 10-base pair deletion between the two promoters. *J Biol Chem* 1995;270:30428–30433. [PubMed: 8530470]
27. Varshavsky A. Electrophoretic assay for DNA-binding proteins. *Methods Enzymol* 1987;151:551–565. [PubMed: 3431454]
28. Fried MG, Liu G. Molecular sequestration stabilizes CAP-DNA complexes during polyacrylamide gel electrophoresis. *Nucl Acids Res* 1994;22:5054–5059. [PubMed: 7800499]
29. Fried MG, Bromberg JL. Factors that affect the stability of protein-DNA complexes during gel electrophoresis. *Electrophoresis* 1997;18:6–11. [PubMed: 9059813]
30. Vossen KM, Fried MG. Sequestration stabilizes *lac* repressor-DNA complexes during gel electrophoresis. *Anal Biochem* 1997;245:85–92. [PubMed: 9025972]
31. Garner MM, Revzin A. Stoichiometry of catabolite activator protein/adenosine cyclic 3',5'-monophosphate interactions at the *lac* promoter of *Escherichia coli*. *Biochemistry* 1982;21:6032–6036. [PubMed: 6295452]
32. Fried MG, Crothers DM. CAP and RNA polymerase interactions with the *lac* promoter: binding stoichiometry and long range effects. *Nucl Acids Res* 1983;11:141–158. [PubMed: 6306561]

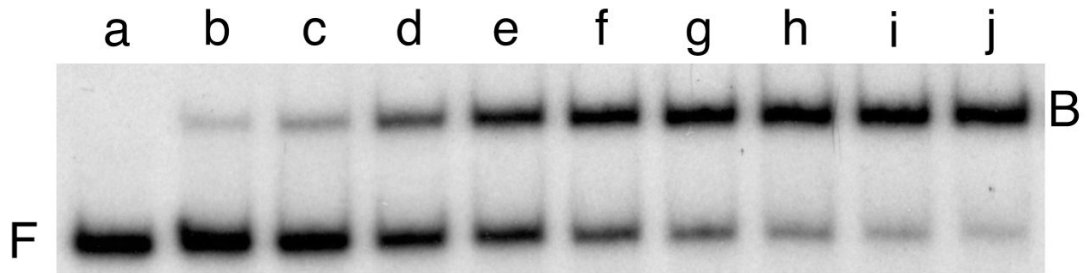
33. Rasimas JJ, et al. DNA-binding mechanism of O<sup>6</sup>-alkylguanine-DNA alkyltransferase. Effects of protein and DNA alkylation on complex stability. *J Biol Chem* 2003;278:7973–7980. [PubMed: 12496275]
34. Ucci JW, Cole JL. Global analysis of non-specific protein-nucleic interactions by sedimentation equilibrium. *Biophys Chem* 2004;108:127–140. [PubMed: 15043926]
35. Brenowitz M, et al. Quantitative DNase footprint titration: a method for studying protein-DNA interactions. *Methods Enzymol* 1986;130:132–181. [PubMed: 3773731]
36. Tullius TD, et al. Hydroxyl radical footprinting: a high resolution method for mapping protein-DNA contacts. *Methods Enzymol* 1987;155:537–559. [PubMed: 2828876]
37. Hendrickson W, Schleif RF. A dimer of AraC protein contacts three adjacent major groove regions of the araI DNA site. *Proc Nat'l Acad Sci U S A* 1985;82:3129–3133.
38. Revzin A. Gel electrophoresis assays for DNA-protein interactions. *BioTechniques* 1989;7:346–355. [PubMed: 2698199]
39. Gerstle JT, Fried MG. Measurement of binding kinetics using the gel electrophoresis mobility shift assay. *Electrophoresis* 1993;14:725–731. [PubMed: 8404816]
40. Papoulas, O. Rapid separation of protein-bound DNA from free DNA using nitrocellulose filters. In: Ausubel, FM.; Brent, R.; Kingston, RE.; Moore, DD.; Seidman, JG.; Smith, JA.; Struhl, K., editors. *Current Protocols in Molecular Biology*. John Wiley & Sons; New York: 1989. p. 12.18.11-12.18.19.
41. Hall, K.; Kranz, J. Nitrocellulose filter binding for determination of dissociation constants. In: Haynes, SR., editor. *RNA Protein Interaction Protocols*. Humana Press; Totowa, NJ: 1999. p. 105-114.
42. Galas DJ, Schmitz A. DNAase footprinting: a simple method of the detection of protein-DNA binding specificity. *Nucl Acids Res* 1978;5:3157–3170. [PubMed: 212715]
43. Riggs AD, et al. The *lac* repressor-operator interaction. 3 Kinetic studies. *J Mol Biol* 1970;53:401–417. [PubMed: 4924006]
44. Woodbury CP Jr, von Hippel PH. On the determination of deoxyribonucleic acid-protein interactions parameters using the nitrocellulose filter-binding assay. *Biochemistry* 1983;22:4730–4737. [PubMed: 6626528]
45. Oehler S, et al. Is nitrocellulose filter binding really a universal assay for protein DNA interactions? *Analytical Biochemistry* 1999;268:330–336. [PubMed: 10075823]
46. Sanger F, et al. Nucleotide sequence of bacteriophage lambda DNA. *J Mol Biol* 1982;162:729–773. [PubMed: 6221115]
47. Whitson PA, Matthews KS. Dissociation of the lactose repressor-operator DNA complex: effects of size and sequence context of operator-containing DNA. *Biochemistry* 1986;25:3845–3852. [PubMed: 3527257]
48. Fried MG, Stickle DF. Ion exchange reactions of proteins during DNA binding. *Eur J Biochem* 1993;218:469–475. [PubMed: 8269936]
49. Stickle DF, Fried MG. Cation binding linked to a sequence-specific CAP-DNA interaction. *Biophys Chem* 2007;126:106–116. [PubMed: 16782261]
50. Beattie KL, et al. Uptake of homologous single-stranded fragments by superhelical DNA. *J Mol Biol* 1977;116:783–803. [PubMed: 592401]
51. Tullius TD. Physical studies of protein-DNA complexes by footprinting. *Annu Rev Biophys Biophys Chem* 1989;18:213–237. [PubMed: 2660824]
52. Brenowitz, M., et al. DNase I footprint analysis of protein-DNA binding. In: Ausubel, FM.; Brent, R.; Kingston, RE.; Moore, DD.; Seidman, JG.; Smith, JA.; Struhl, K., editors. *Current Protocols in Molecular Biology*. John Wiley & sons; New York: 1987. p. 12.14.11-12.14.16.
53. Sclavi B, et al. Time-resolved synchrotron X-ray “footprinting”, a new approach to the study of nucleic acid structure and function: application to protein-DNA interactions and RNA folding. *J Mol Biol* 1997;266:144–159. [PubMed: 9054977]
54. Shcherbakova I, et al. Fast Fenton footprinting: a laboratory-based method for the time-resolved analysis of DNA, RNA and proteins. *Nucleic Acids Research* 2006;34:e48. [PubMed: 16582097]
55. Naritsin DB, Lyubchenko YL. Melting of oligodeoxynucleotides with various structures. *J Biomol Struct Dyn* 1991;8:813–825. [PubMed: 2059341]

56. Vallone PM, Benight AS. Thermodynamic, spectroscopic, and equilibrium binding studies of DNA sequence context effects in four 40 base pair deoxyoligonucleotides. *Biochemistry* 2000;39:7835–7846. [PubMed: 10869190]
57. Olmsted MC, et al. Monte Carlo description of oligoelectrolyte properties of DNA oligomers: range of the end effect and the approach of molecular and thermodynamic properties to the polyelectrolyte limits. *Proc Natl Acad Sci USA* 1989;86:7766–7770. [PubMed: 2813356]
58. Maxam A, Gilbert WS. A new method for sequencing DNA. *Proc Natl Acad Sci USA* 1977;74:560–565. [PubMed: 265521]
59. Forwood JK, Jans DA. Quantitative analysis of DNA-protein interactions using double-labeled native gel electrophoresis and fluorescence-based imaging. *Electrophoresis* 2006;27:3166–3170. [PubMed: 16915571]
60. Kang J, Lee MS, Gorenstein DG. Quantitative analysis of chemiluminescence signals using a cooled charge-coupled device camera. *Anal Biochem* 2005;345:66–71. [PubMed: 16125123]
61. Li Y, et al. A modified quantitative EMSA and its application in the study of RNA-protein interactions. *J Biochem Biophys Methods* 2004;60:85–96. [PubMed: 15262445]
62. Man TK, Stormo GD. Non-independence of Mnt repressor-operator interaction determined by a new quantitative multiple fluorescence relative affinity (QuMFRA) assay. *Nucleic Acids Res* 2001;29
63. Record, MT.; Mossing, MC. Physical-chemical origins of stability, specificity, and control of protein-DNA interactions. In: Reznikoff, WS., editor. *RNA Polymerase and the Regulation of Transcription*. Elsevier; New York, NY: 1987. p. 61-83.
64. Carey J. Gel retardation at low pH resolves *trp* repressor-DNA complexes for quantitative study. *Proc Natl Acad Sci USA* 1988;85:975–979. [PubMed: 3277190]
65. Garner MM, Rau DC. Water release associated with specific binding of *gal* repressor. *EMBO J* 1995;14:1257–1263. [PubMed: 7720716]
66. Vossen KM, et al. The role of macromolecular hydration in the binding of the *E. coli* cyclic AMP receptor to DNA. *Biochemistry* 1997;36:11640–11647. [PubMed: 9305953]
67. Fried MG, Crothers DM. Equilibrium studies of the cyclic AMP receptor protein-DNA interaction. *J Mol Biol* 1984;172:241–262. [PubMed: 6319715]
68. Bell CE. Structure and mechanism of *Escherichia coli* RecA ATPase. *Molecular Microbiology* 2005;58:358–366. [PubMed: 16194225]
69. Chi P, et al. Roles of ATP binding and ATP hydrolysis in human Rad51 recombinase function. *DNA Repair* 2006;5:381–391. [PubMed: 16388992]
70. Panagiotidis CA, et al. Polyamines alter sequence-specific DNA-protein interactions. *Nucleic Acids Res* 1995;3:1800–1809. [PubMed: 7784186]
71. Maniatis T, Efstratiadis A. Fractionation of low molecular weight DNA or RNA in polyacrylamide gels containing 98% formamide or 7M urea. *Method Enzymol* 1980;65:299–305.
72. Maxam AM, Gilbert W. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol* 1980;65:449–560. [PubMed: 6246365]
73. Ogden RC, Adams DA. Electrophoresis in agarose and acrylamide gels. *Methods Enzymol* 1987;152:55–61. [PubMed: 3657589]
74. Berman J, et al. An agarose gel electrophoresis assay for the detection of DNA binding activities in yeast cell extracts. *Methods Enzymol* 1987;155:528–537. [PubMed: 3323822]
75. Dignam GD, et al. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* 1983;11:1495–1487.
76. Manley JL, et al. In vitro transcription: whole-cell extract. *Methods Enzymol* 1983;101:568–582. [PubMed: 6193397]
77. Farrell, RE. *RNA Methodologies: A Laboratory Guide For Isolation And Characterization*. Academic Press; San Diego: 1998. p. 221-244.
78. Studier FW. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *J Mol Biol* 1973;79:237–248. [PubMed: 4760132]
79. Crothers DM, Drak J. Global features of DNA structure by comparative gel electrophoresis. *Methods in Enzymology* 1992;212:46–71. [PubMed: 1518459]

80. Pearson L, et al. Footprinting RNA-protein complexes following gel retardation assays: application to the R-17-procoat-RNA and tat-TAR interactions. *Nucl Acids Res* 1994;22:2255–2263. [PubMed: 8036153]
81. Laue TM, Stafford WF III. Modern applications of analytical ultracentrifugation. *Annu Rev Biophys Biomol Struct* 1999;28:75–100. [PubMed: 10410796]
82. Lebowitz J, et al. Modern analytical ultracentrifugation in protein science: a tutorial review. *Protein Sci* 2002;11:2067–2079. [PubMed: 12192063]
83. Daugherty MA, Fried MG. Analysis of transcription factor interactions at sedimentation equilibrium. *Methods Enzymol* 2003;370:349–369. [PubMed: 14712659]
84. Kristie TM, Roizman B. Alpha 4, the major regulatory protein of herpes simplex virus type 1, is stably and specifically associated with promoter-regulatory domains of alpha genes and of selected other viral genes. *Proc Natl Acad Sci U S A* 1986;83:3218–3222. [PubMed: 3010285]
85. Granger-Schnarr M, et al. Specific protein-DNA complexes: immunodetection of the protein component after gel electrophoresis and Western blotting. *Anal Biochem* 1988;174:235–238. [PubMed: 3064651]
86. Chen H, Chang GD. Simultaneous immunoblotting analysis with activity gel electrophoresis in a single polyacrylamide gel. *Electrophoresis* 2001;22:1894–1899. [PubMed: 11465485]
87. Woo AJ, et al. A proteomics approach for the identification of DNA-binding activities observed in the electrophoretic mobility shift assay. *Mol Cellular Proteomics* 2002;1:472–478.
88. Stead JA, et al. The identification of nucleic acid-interacting proteins using a simple proteomics-based approach that directly incorporates the electrophoretic mobility shift assay. *Mol Cell Proteomics* 2006;5:1697–1702. [PubMed: 16845145]
89. Stead, JA.; McDowell, KJ. *Nature Protocols*. 2007. Two-dimensional gel electrophoresis for identifying proteins that bind DNA or RNA. Submitted
90. Vossen KM, et al. The mechanism of CAP-lac repressor binding cooperativity at the *E. coli* lactose promoter. *J Mol Biol* 1996;255:44–54. [PubMed: 8568874]
91. Huang CY. Determination of binding stoichiometry by the continuous variation method: the Job plot. *Methods in Enzymology* 1982;87:509–525. [PubMed: 7176926]
92. Crothers DM, et al. DNA bending in protein-DNA complexes. *Methods Enzymol* 1991;208:118–146. [PubMed: 1779833]
93. Senear DF, Brenowitz M. Determination of binding constants for cooperative site-specific protein-DNA interactions using the gel mobility-shift assay. *J Biol Chem* 1991;266:13661–13671. [PubMed: 1856200]
94. Senear DF, et al. Effects of anomalous migration and DNA to protein ratios on resolution of equilibrium constants from gel mobility-shift assays. *Electrophoresis* 1993;14:704–712. [PubMed: 8404813]
95. Hope IA, Struhl K. GCN4 protein, synthesized in vitro, binds HIS3 regulatory sequences: implications for general control of amino acid biosynthetic genes in yeast. *Cell* 1985;43:177–188. [PubMed: 3907851]
96. Bain DL, Ackers GK. A quantitative cryogenic gel-shift technique for analysis of protein-DNA binding. *Anal Biochem* 1998;258:240–245. [PubMed: 9570836]
97. Taylor IC, et al. Facilitated binding of GAL4 and heat shock factor to nucleosomal templates: differential function of DNA-binding domains. *Genes Dev* 1991;5:1285–1298. [PubMed: 2065977]
98. Li Q, Wrangé O. Assays for transcription factor access to nucleosomal DNA. *Methods* 1997;12:96–104. [PubMed: 9169199]
99. Hope IA, Struhl K. GCN4, a eukaryotic transcriptional activator protein, binds as a dimer to target DNA. *EMBO J* 1987;9:2781–2784. [PubMed: 3678204]
100. Blackwell TK, Wientraub H. Differences and similarities in DNA-binding preferences of MyoD and E2A protein complexes revealed by binding site selection. *Science* 1990;250:1104–1110. [PubMed: 2174572]
101. Park SH, Raines RT. Green fluorescent protein as a signal for protein-protein interactions. *Protein Sci* 1997;6:2344–2349. [PubMed: 9385636]

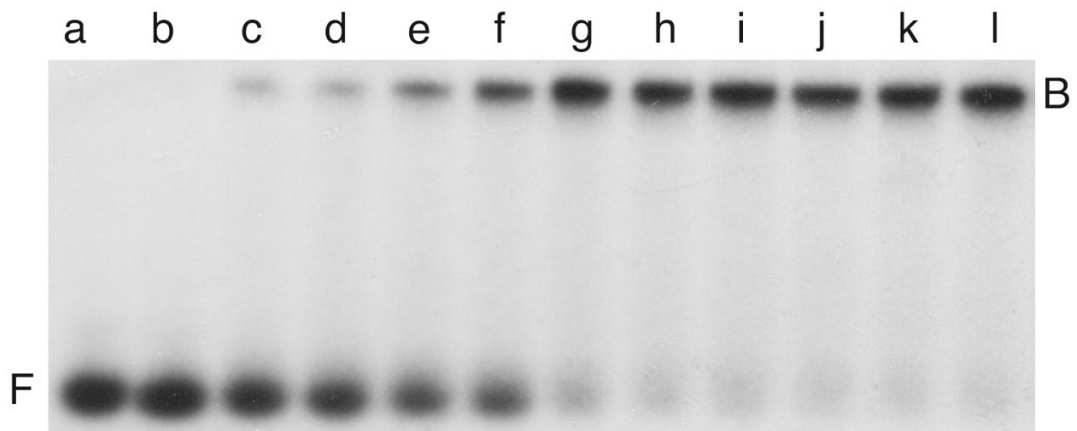


102. Cooney M, et al. Site-specific oligonucleotide binding represses transcription of the human c-myc gene in vitro. *Science* 1988;241:456–459. [PubMed: 3293213]
103. Ferber MJ, Maher LJ. Quantitating oligonucleotide affinities for duplex DNA: footprinting vs electrophoretic mobility shift assays. *Anal Biochem* 1997;244:312–320. [PubMed: 9025948]
104. Konarska MM, Sharp PA. Electrophoretic separation of complexes involved in the splicing of precursors to mRNAs. *Cell* 1986;46:845–855. [PubMed: 2944598]
105. Kang J, et al. Chemiluminescence-based electrophoretic mobility shift assay of heparin-protein interactions. *Anal Biochem* 2006;349:156–158. [PubMed: 16352290]
106. Boffini A, Prentki P. Identification of protein binding sites in genomic DNA by two-dimensional gel electrophoresis. *Nucleic Acids Res* 1991;19:1369–1374. [PubMed: 1827523]
107. Jett SD, Bear DG. Snapshot blotting: transfer of nucleic acids and nucleoprotein complexes from electrophoresis gels to grids for electron microscopy. *Proc Nat'l Acad Sci USA* 1994;91:6870–6874.
108. Lynch TW, et al. Dissecting the molecular origins of specific protein-nucleic acid recognition: hydrostatic pressure and molecular dynamics. *Biophys J* 2002;82:93–98. [PubMed: 11751298]
109. Hudson JM, et al. Effects of anions on the binding of the cyclic AMP receptor protein to the lactose promoter. *Eur J Biochem* 1993;212:539–548. [PubMed: 8383049]
110. Stickle DF, et al. Analysis of the thermodynamic linkage of DNA binding and ion binding for dimeric and tetrameric forms of the *lac* repressor. *Eur J Biochem* 1994;226:869–876. [PubMed: 7813477]
111. Fried MG, et al. Role of Macromolecular Hydration in Lac Repressor-DNA Interactions. *J Biol Chem* 2002;277:50676–50682. [PubMed: 12379649]
112. Sidorova NY, Rau DC. Differences between EcoRI nonspecific and “star” sequence complexes revealed by osmotic stress. *Biophys J* 2004;87:2564–2576. [PubMed: 15454451]
113. Robinson CR, Sligar SG. Changes in solvation during DNA binding and cleavage are critical to altered specificity of the Eco RI endonuclease. *Proc Nat Acad Sci USA* 1998;95:2186–2191. [PubMed: 9482860]
114. Lynch TW, Sligar SG. Macromolecular hydration changes associated with BamHI binding and catalysis. *J Biol Chem* 2000;275:30561–30565. [PubMed: 10878000]



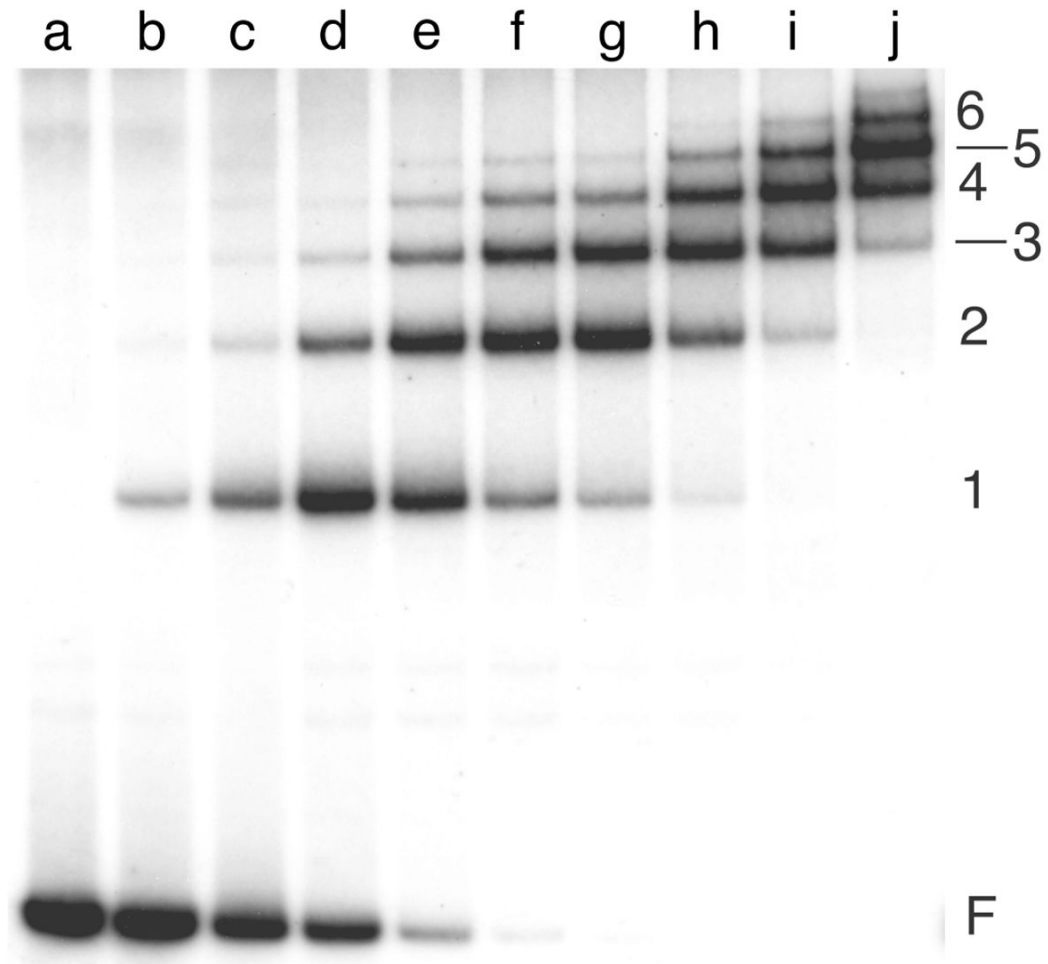
**Figure 1. Titration of a 214 bp *lac* promoter DNA fragment with *E. coli* CAP protein**

Sample compositions are given in Table 4. The complex consists of a single molecule of CAP bound predominantly to the highest affinity CAP site in the *lac* promoter (CAP site 1<sup>49, 66</sup>). Electrophoresis was carried out with a 10% w/v polyacrylamide (75:1 acrylamide:bisacrylamide) gel, cast and run in the Tris-acetate EDTA buffer described in the protocol shown in Table 5. Cyclic AMP (final concentration 20  $\mu$ M) was added to the electrophoresis running buffer. Pre-electrophoresis ensured that the cAMP was distributed throughout the gel before the samples were loaded. Band designations: F, free DNA; B, bound DNA (i.e., protein-DNA complex).



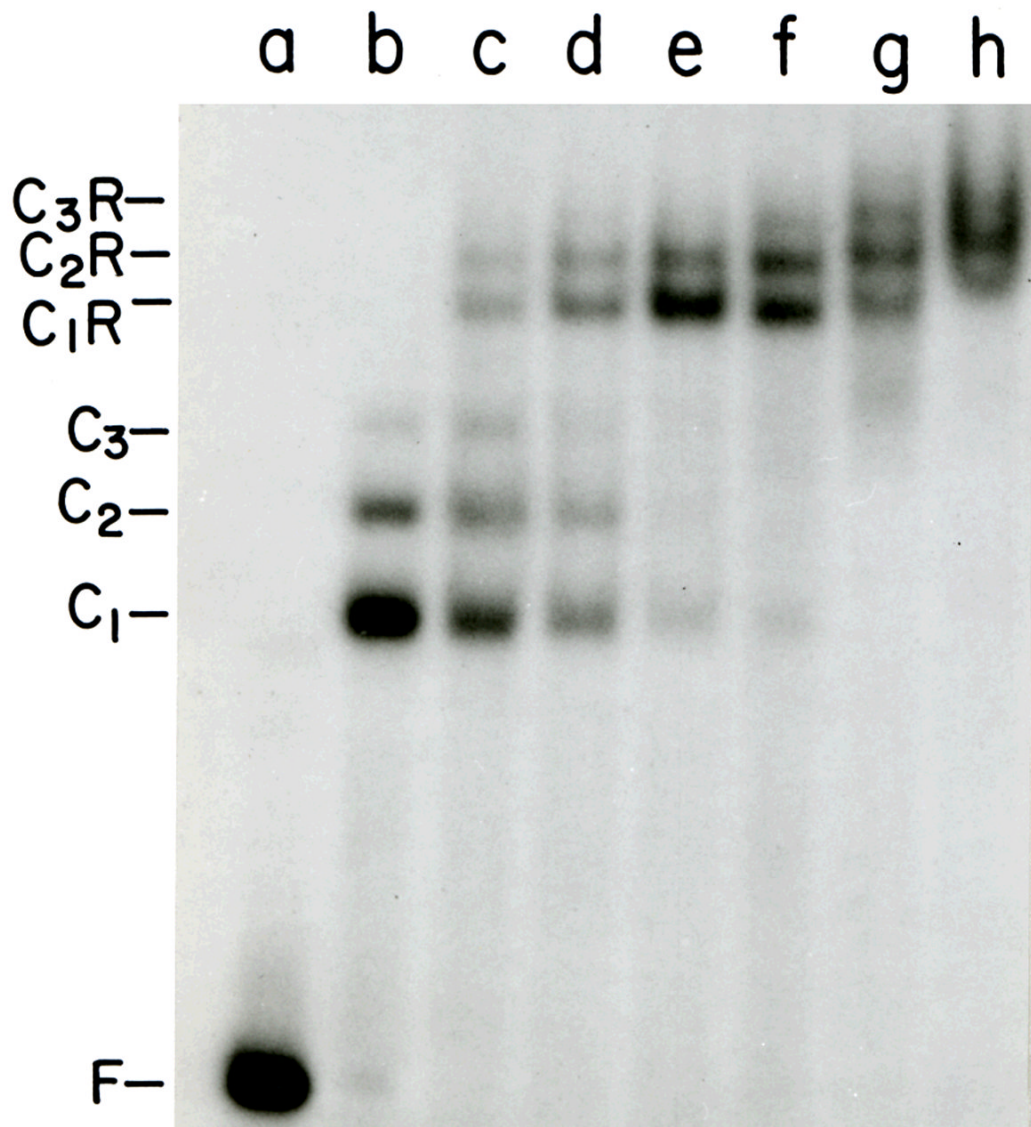
**Figure 2. Titration of a 16-residue single-stranded DNA with human AGT protein**

All samples contained  $8.7 \times 10^{-7}$  M DNA; samples b-l contained in addition 0.57, 1.14, 1.72, 2.29, 2.86, 3.44, 4.01, 4.58, 5.16, 5.73 and  $6.60 \times 10^{-6}$  M AGT protein, respectively. The binding buffer contained 10 mM Tris (pH 7.6 at 20°C), 50 mM KCl, 1mM dithiothreitol, 10  $\mu$ g/mL bovine serum albumin. Samples were resolved on a 10% w/v polyacrylamide gel cast and run in 40 mM Tris-Acetate, 2 mM EDTA, 50 mM KCl. Band designations: F, free DNA; B, protein-DNA complex. Continuous variation EMSA and analytical ultracentrifugation analyses support the conclusion that the complex has a 4:1 stoichiometry<sup>19, 33</sup>.



**Figure 3. Titration of a 203 bp *lac* promoter DNA with *E. coli* lactose repressor**

All samples contained  $1.91 \times 10^{-9}$  M DNA; samples b-j contained 0.08, 0.16, 0.32, 0.48, 0.61, 0.83, 0.99, 1.32,  $1.66 \times 10^{-8}$  M repressor protein, respectively. The binding buffer contained 10 mM Tris (pH 8 at 20°C), 1 mM EDTA, 50 mM KCl, 100  $\mu$ g/mL bovine serum albumin, 5% v/v glycerol. Samples were resolved on a 5% w/v polyacrylamide gel cast and run at room temperature ( $20 \pm 2^\circ\text{C}$ ) in 45 mM Tris-borate (pH 7.8), 2.5 mM EDTA. Electrophoretic species are designated F, free DNA or numbered (1–6) to indicate the repressor:DNA ratio of the corresponding complex<sup>7</sup>. Under these conditions, repressor binds non-specific sites as well as its specific operator sequences.



**Figure 4. Titration of 1:1, 2:1 and 3:1 CAP-*lac* promoter complexes with *lac* repressor**

All samples contained a 214 bp *E. coli lac* promoter-operator DNA<sup>90</sup> ( $3.7 \times 10^{-10}$ M). Samples b-h contained CAP protein ( $7.1 \times 10^{-9}$ M). Samples c-h contained *lac* repressor at 0.7, 1.5, 2.2, 2.9, 3.6 and  $7.3 \times 10^{-9}$ M, respectively. The binding buffer was 10 mM Tris (pH 8.0 at 20°C), 1 mM EDTA, 50 mM KCl, 20  $\mu$ M cAMP. Electrophoresis was carried out at room temperature in a 5% w/v polyacrylamide gel run in 45 mM Tris-borate (pH 8.0), 2 mM EDTA, 20  $\mu$ M cAMP. Symbols: F, free DNA; C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub>, complexes with 1, 2 and 3 CAP dimers bound per DNA molecule, respectively; C<sub>1</sub>R, C<sub>2</sub>R and C<sub>3</sub>R, complexes with one repressor tetramer and 1, 2 and 3 CAP dimers bound per DNA molecule, respectively.

**Table 1**  
EMSA Variants

Method and Reference(s)	Purpose
<b>1-dimensional methods</b>	
Time course <sup>7, 39</sup>	Measurement of association and/or dissociation kinetics
Double-label assays <sup>31, 32</sup>	Determination of binding stoichiometry
Continuous variation <sup>33, 91</sup>	Determination of binding stoichiometry
Circular permutation <sup>92</sup>	Detection of DNA bending
Phased bends analysis <sup>79</sup>	Determination of direction of a protein-induced bend with respect to a standard bend locus
Binding partition analysis <sup>3, 12, 18, 93, 94</sup>	Evaluation of binding cooperativity for nucleic acids with multiple protein binding sites; comparison of binding affinities of one protein for several target sequences, determination of the effect of one protein on binding affinity of a second.
Reverse EMSA <sup>95</sup>	Detection of nucleic acid binding, measurement of binding affinity. In this assay, the tracer species is the protein. In the classical EMSA the nucleic acid is the tracer species.
Topoisomer EMSA <sup>22</sup>	Detection of complexes formed with supercoiled DNA
Cryogenic-EMSA <sup>96</sup>	Detection of labile complexes
Antibody supershift <sup>84</sup>	Identification of protein(s) carrying a specific epitope in mobility-shifted complex(es)
Nucleosome shift <sup>97,98</sup>	Detection of protein(s) that bind nucleosomes
Heterogeneous subunit assay <sup>99</sup>	Determination of whether binding activity is due to a protein monomer or a protein oligomer
Binding site selection <sup>100</sup>	Selection of oligonucleotides containing a specific protein binding site from an ensemble of partially randomized sequences, allowing subsequent amplification and sequencing
Protein-protein <sup>101</sup>	Detection of protein interactions in the absence of nucleic acids
DNA-DNA and RNA-RNA <sup>102,103,104</sup>	Detection of nucleic acid interactions in the absence of proteins
Protein-oligosaccharide <sup>105</sup>	Detection of protein binding to charged oligosaccharides
<b>2-dimensional methods</b>	
EMSA followed by high temperature electrophoresis <sup>106</sup>	Identification of DNA fragments containing specific binding sites for a given protein
EMSA as an adjunct to footprinting <sup>37</sup>	Reduction of signal from free nucleic acid in footprint pattern
EMSA followed by electron microscopy <sup>107</sup>	Characterization of conformation
EMSA followed by SDS-polyacrylamide gel electrophoresis with Western blot detection <sup>85, 86</sup>	Identification of unknown binding proteins
EMSA followed by MS <sup>87, 88</sup>	Identification of unknown binding proteins

**Table 2**

Representative Ranges of Conditions for EMSA Using Polyacrylamide Gels Unless indicated, conditions refer to sample equilibration prior to electrophoresis.

Quantity	Range	Comment	Reference
Temperature (T)	$0^{\circ}\text{C} \leq T \leq 60^{\circ}\text{C}$	Temperature range applies to electrophoresis as well as sample equilibration. The observed range was limited by the incubator used, not the assay. This range applies to electrophoresis using polyacrylamide gels only. As a result of changes in agarose gel properties with temperature our tests have not exceeded $37^{\circ}\text{C}$ .	M. Fried, unpublished result
Pressure (P)	$\text{ambient} \leq P \leq 500 \text{ bar}$	Sample and gel incubated in pressure chamber, electrophoresis performed under pressure.	108
pH	$4.0 \leq \text{pH} \leq 9.5$	Care was taken to match conductivity of sample and electrophoresis buffer.	M. Fried, unpublished result
Monovalent salt ( $\text{M}^+\text{X}^-$ )	$1 \text{ mM} \leq [\text{M}^+\text{X}^-] \leq 300 \text{ mM}$	Care was taken to match conductivity of sample and electrophoresis buffer. High-salt experiments were run in cold room to limit heating of gel. Buffer was circulated between cathodic and anodic reservoirs during electrophoresis of samples containing $\leq 20 \text{ mM}$ salt. <b>CAUTION!</b> Electrolysis of chloride salt solutions generates chlorine gas. Where the chance of chlorine generation exists, electrophoresis should be performed in a well-ventilated area and preferably a fume hood.	7:109:110:49
Oligovalent ions	[Calcium acetate] $\leq 20 \text{ mM}$ [Magnesium acetate] $\leq 20 \text{ mM}$ [Spermidine chloride] $\leq 10 \text{ mM}$ [Potassium phosphate] $\leq 300 \text{ mM}$ [Sodium borate] $\leq 200 \text{ mM}$ [Potassium sulfate] $\leq 300 \text{ mM}$	Approximate limits, obtained for each salt separately in studies of the CAP- and <i>lac</i> repressor-DNA interactions. Care was taken to match conductivity of sample and electrophoresis buffer. High-salt experiments were run in cold room to limit heating of gel. Limits reflect the stability of these protein-DNA complexes, not the resolution of the electrophoretic process.	109 M. Fried, unpublished result.
Zwitterions (Z) (e.g., glycine, proline, betaine)	$[\text{Z}] \leq 2\text{M}$	Solutes used in samples for osmotic stress experiments.	65:66:111:112
Neutral solutes (N) (e.g., glycerol, ethylene glycol, sucrose)	$[\text{N}] \leq 2 \text{ M}$	Sometimes added to samples to aid loading of gels. Used in samples for osmotic stress experiments. Use in gel buffer can stabilize some complexes during electrophoresis.	65:66:113: 114:111:112
Neutral polymers (e.g., Polyethylene glycol 8000, linear polyacrylamide)	[polymer] $\leq 10\% \text{ w/v}$	Limited by difficulty in loading gels with viscous samples. Electrophoretic resolution was unaffected, even at the highest neutral polymer concentrations.	29: 66
Reducing agents	[dithiothreitol] $\leq 10 \text{ mM}$ [2-mercaptoethanol] $\leq 10\text{mM}$ [thioglycolic acid] $\leq 5 \text{ mM}$	Dithiothreitol and 2-mercaptoethanol were added to samples only. Due to its negative charge, thioglycolate can be included in the gel running buffer and will migrate into the gel to ensure the presence of a reducing environment within the gel matrix.	33 M. Fried, unpublished result.

Table 3

## Troubleshooting

Problem	Possible Causes	Possible Remedies
No bands visible after electrophoresis	Low nucleic acid concentration	Verify nucleic acid concentration. Repeat experiment with appropriate concentration of nucleic acid.
	Inefficient labeling	Check reaction components for concentration and enzyme activity
	Nucleic acid degradation	If sample is radioactive, verify that nucleic acid is intact DNA by running a sequencing gel. If nuclease activity is suspected, treat buffers with diethyl pyrocarbonate. Exclude divalent cations wherever possible. Commercially available RNase inhibitors are useful to protect RNA substrates. Commercially-available phosphatase inhibitors can prevent substrate dephosphorylation.
All bands are smeared or streaked	Uneven gel polymerization	Use fresh gel components. Degas thoroughly before polymerization. If polymerization interfered with casting gel, reduce TEMED concentration. If gel required >1h to polymerize, increase ammonium persulfate concentration.
	Excessive gel heating	Check concentrations of gel and running buffer. If they are correct, reduce voltage during electrophoresis.
	Sample conductivity too high.	Reduce salt concentration in nucleic acid or sample buffer.
Free nucleic acid migrates normally. Nucleic acid mobility unchanged in presence of protein	Low protein concentration	Verify protein concentration. Use larger volumes of protein stock or more concentrated stock in preparing EMSA samples.
	Protein is inactive	Run SDS-polyacrylamide gel electrophoresis to rule out degradation. If the binding protein is an enzyme, test for activity. Test higher concentrations of protein to detect residual binding activity. A new, more active preparation of protein may be necessary.
	Protein is negatively charged and co-migrates with nucleic acid	Check migration of protein alone under EMSA conditions. If protein co-migrates with nucleic acid, reduce pH of binding and electrophoresis buffers <sup>64</sup> .
Free nucleic acid migrates normally. No nucleic acid detectable in samples containing protein.	Nucleic acid degradation	Verify that nucleic acid is intact. If nuclease activity is suspected, treat glassware and buffers with diethyl pyrocarbonate. Exclude divalent cations wherever possible. Use commercial RNase and phosphatase inhibitors.
	Binding occurred but complexes dissociated during electrophoresis. Liberated nucleic acid is too diffuse to detect.	Minimize gel running times; use more concentrated gel; include stabilizing solutes in gel buffer; reduce salt concentration in binding and electrophoresis buffers to increase electrostatic stabilization; lower electrophoresis temperature (run gel in cold room); reduce or eliminate competing nucleic acid (this can be added back with care once a useful binding signal is obtained).
Free nucleic acid migrates normally. Complex bands smeared or streaky	Binding occurred but complexes dissociated in gel during electrophoresis.	Minimize gel running times; use more concentrated gel; include stabilizing solutes in gel buffer; reduce salt concentration in binding and electrophoresis buffers to increase electrostatic stabilization; lower electrophoresis temperature (run gel in cold room); reduce or eliminate competing nucleic acid.
	Binding occurred but complexes dissociated in the well prior to electrophoresis.	Minimize interval that sample is in well before electrophoresis. Complexes may be destabilized by component(s) of running buffer. If so they may be more stable in gel and running buffers that more closely resemble the composition of binding buffer.
	Binding occurred but complexes dissociated in the well during electrophoresis.	To minimize gel "dead time", use smallest sample volume possible; conduct electrophoresis at high voltage (~50 V/cm) until samples enter the gel, then reduce to ~10V/cm.
	Binding occurred but samples containing protein are too salty.	Reduce salt concentration in protein stock and/or in binding buffer.
Free band is sharp, complex band(s) are broad and indistinct	Heterogeneous protein.	Multiple species may be due to post-translational modification or to partial degradation without loss of binding activity.
Complex and free bands are broad and indistinct	Sample zone is too large (measured from top of sample to bottom of well) at the start of electrophoresis	Reduce sample volume. Increase density of sample (e.g., increase glycerol concentration) to facilitate gel loading. Minimize time between loading and electrophoresis



<b>Problem</b>	<b>Possible Causes</b>	<b>Possible Remedies</b>
Nucleic acid stuck in well, no free species visible	Electrophoresis period too long	Reduce run-time
	Nucleic acid degradation	Verify that nucleic acid is intact. If nuclease activity is suspected, treat extracts and buffers with diethyl pyrocarbonate. Exclude divalent cations wherever possible. Use RNase and phosphatase inhibitors.
	Protein/nucleic acid ratio is too high	Reduce the concentration of protein or increase the concentration of unlabeled nonspecific competitor
	Protein is aggregated	Change binding conditions to improve protein solubility. Possible modifications: add solutes that stabilize folded (compact) forms of proteins (e.g., glycerol); keep protein stocks and binding reactions at ice temperature; avoid freeze-thaw cycles with protein stocks; include non-ionic detergents in protein storage buffer and/or binding buffer
	Free nucleic acid and complexes are too large for gel system	Try lower percentage polyacrylamide or reduce the acrylamide/bisacrylamide ratio. Test agarose gel as alternative to polyacrylamide.

**Table 4**

Composition of typical gel polymerization mixtures

Components	Volume		
	5% w/v gel	10% w/v gel	15% w/v gel
<b>Combine and perform steps in top-down order</b>			
10X Tris acetate EDTA electrophoresis buffer. 400 mM Tris, 25 mM EDTA, brought to pH 7.8 with acetic acid	2.0 mL	2.0 mL	2.0 mL
40% w/v acrylamide-bisacrylamide stock solution	2.5 mL	5.0 mL	7.5 mL
H <sub>2</sub> O	15.5 mL	13.0 mL	10.5 mL
Ammonium persulfate	0.05 g	0.05 g	0.05 g
Degas under mild vacuum 15 min with stirring. A stoppered side-arm flask connected to a water aspirator or “house vacuum” works well.			
Start polymerization by adding TEMED ( <i>N,N,N',N'</i> -Tetramethylethylenediamine)	12 μL	12 μL	12 μL

**! CAUTION** Always interpose a trap between the side-arm flask and the vacuum source, to prevent contamination of the vacuum source with acrylamide.

**Table 5**  
Composition of binding reactions for the assay shown in Fig. 1.

Component	Sample									
	a	b	c	d	e	f	g	h	i	j
	Volumes in $\mu\text{L}$									
10X Binding buffer: 100 mM Tris (pH 7.5 at 20°C), 10 mM EDTA, 1 M KCl, 1 mM DTT, 50% v/v glycerol, 0.10 mg/mL BSA	3	3	3	3	3	3	3	3	3	3
$^{32}\text{P}$ DNA: 214 bp <i>lac</i> promoter-operator fragment $^{48}$ , $1.25 \times 10^{-8}\text{M}$ , ~4000 cpm/ $\mu\text{L}$ .	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
200 $\mu\text{M}$ cAMP	3	3	3	3	3	3	3	3	3	3
$\text{H}_2\text{O}$	22.5	21.5	20.5	18.5	16.5	14.5	12.5	7.5	20.5	18.5
DNA-binding protein:										
<i>E. coli</i> CAP, $1.4 \times 10^{-8}\text{M}$	0	1	2	4	6	8	10	15	2	4
<i>E. coli</i> CAP, $1.4 \times 10^{-7}\text{M}$										