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# A Detailed and Radioisotope-free Protocol for Electrophoretic Mobility Shift Assay (EMSA)

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**[Abstract]** To comprehensively characterize the functions of a transcription factor (TF), it is required to analyze the interaction of this TF with its targeted loci. Several methods such as β-glucuronidase (GUS) or luciferase reporter, yeast one-hybrid (Y1H), chromatin-immunoprecipitation (ChIP), and electrophoretic mobility shift assay (EMSA) assays have been developed. Of these, EMSA is an *in vitro* method which can prove the direct interaction between TF and targeted DNA fragment. In the present protocol, DNA probes are labeled with Biotin. Therefore, it is safer for researchers when they do not need to use radioisotope-labeled probes. In addition, this protocol is to provide a detailed procedure for a successful EMSA assay. The interested recombinant protein can be mixed with putative-targeted DNA probes for a binding reaction at room temperature (25 °C). Afterward, the reaction mixture can be run in a native polyacrylamide gel and transferred to a positively charged nylon membrane. Finally, the results can be detected and visualized via the biotin-streptavidin chemiluminescence.
**Keywords:** DNA-protein interaction, DNA binding protein, Electrophoretic mobility shift assay, EMSA, Transcription factor

**[Background]** EMSA is a powerful method to detect the direct interaction between a protein (*e.g.*, transcription factor) and a DNA fragment (Chen, 2011). Typically, a protein-bound DNA probe moves slower than the free-DNA probes in a gel. This protein-DNA complex can be observed as a shifted band and the free-DNA probes move faster toward the bottom of the gel. Based on this characteristic, the DNA probes can be labeled with a radioactive isotope [*e.g.*, Phosphorus-32 ($^{32}$P)] or Biotin and the shifted band can be visualized via autoradiography or chemiluminescence, respectively. Several previous EMSA protocols have been published (Hellman and Fried, 2007; Chen, 2011). In these protocols, the DNA probes were labeled with a radioisotope, $^{32}$P (Hellman and Fried, 2007; Chen, 2011). The use of radioisotope is not only dangerous for researchers but also requires specific laboratory infrastructure which is strictly controlled for safety. Here, a detailed and radioisotope-free protocol for EMSA assay is described. This protocol was successfully applied to test the binding of different transcription factors to their targeted DNA fragments in my lab (data not shown).

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## Materials and Reagents

1. 1.5 ml micro-tubes (Eppendorf, catalog number: 0030121589)
2. Positively charged nylon membrane (GE Healthcare Amersham™ Hybond™-N+) (Thermo Fisher Scientific, catalog number: 45-000-927)
3. Interested recombinant proteins
4. Labeled and unlabeled (3′-end Biotin) oligo DNA probes (sense and anti-sense)
   *Note: In this protocol, the length of oligos is 40 bp. The biotin is only added to 3′-end of sense oligo. These oligos can be ordered from a local company and it also provides a service which can label oligo with biotin. If there is no such that service, the Pierce™ Biotin 3′ End DNA Labeling Kit (Thermo Fisher Scientific, catalog number: 89818) can be considered. In addition, it is better to include one more EMSA assay (for negative control) with a non-targeted DNA probe.*
5. Tris pH 7.5 (1 M) (Thermo Fisher Scientific, catalog number: 15567027)
6. NaCl (Sigma-Aldrich, catalog number: S7653)
7. KCl (Sigma-Aldrich, catalog number: P9333)
8. DTT (Dithiothreitol) (Sigma-Aldrich, catalog number: 3483-12-3)
9. Glycerol (Sigma-Aldrich, catalog number: G5516)
10. $MgCl_2$ (Sigma-Aldrich, catalog number: M8266)
11. Poly(dI.dC) (Thermo Fisher Scientific, catalog number: 20148E)
    *Note: The synthetic polymer poly(dI.dC) which can function as a non-specific competitor is added to reduce the nonspecific interaction.*
12. NP-40 (Thermo Fisher Scientific, catalog number: 28324)
13. 30% Acrylamide/Bis Solution, 29:1 (Bio-Rad, catalog number: 1610156)
14. TEMED (N,N,N',N'-tetramethylethylenediamine) (Bio-Rad, catalog number: 161-0801)
15. APS (ammonium persulfate) (Sigma-Aldrich, catalog number: A3678)
16. 6x DNA Gel Loading Dye (Thermo Fisher Scientific, catalog number: R0611)
17. Chemiluminescent Nucleic Acid Detection Module Kit (Thermo Fisher Scientific, catalog number: 89880)
18. Tris base
19. Boric acid
20. EDTA
21. 1 M and 100 mM DTT (see Recipes)
22. 10x TBE buffer (see Recipes)

## Equipment

1. Centrifuge (*e.g.*, Eppendorf, model: 5418R)
2. Heat-block (*e.g.*, Eppendorf, model: Eppendorf ThermoMixer® C)

3. -20 °C freezer
4. Vertical electrophoresis apparatus (*e.g.*, Mini-PROTEAN® system, Bio-Rad, US)
5. Gel transferring apparatus (*e.g.*, Mini Trans-Blot® system, Bio-Rad, US)
6. UV-cross-linker (*e.g.*, Stratalinker® UV Crosslinker)
7. Dancer orbital shaker (*e.g.*, Phoenix, model: RS-OS 10/20)
8. Bio-imaging system equipped with a charge-coupled device (CCD)-camera (*e.g.*, ChemiDoc, Bio-Rad, US)

## Procedure

A. Double-strand DNA probe preparation
   1. Mix the single-strand sense and antisense oligos (100 μM) (ratio 1:1) in a micro-tube. In this step, there are two individual reactions for labeled (3′-end-biotin sense oligo + antisense oligo) and unlabeled (sense oligo + antisense oligo) probes.
      *Note: Basically, the oligo can be delivered in dried form (in a micro-tube). Based on the amount of dried oligo and the company's instruction, add molecular biology-grade water (or Milli-Q purified water) to each oligo tube to obtain the final concentration (100 μM).*
   2. Place the micro-tube in a heat-block at 95 °C for 5 min.
   3. Afterward, turn off the heat-block. Remove the metal rack containing the micro-tube and let all cool down at room-temperature.
      *Note: At this step, an alcohol thermometer can be placed in the metal rack to track the decrease of the temperature. The double-strand DNA can be used when the temperature is around 25 °C.*
   4. Aliquot and add molecular biology-grade water to dilute the labeled DNA probes to 50 nM (1x) and unlabeled DNA probes to 2,500 nM (50x), 5,000 nM (100x), 10,000 nM (200x), and 15,000 nM (300x).
   5. Store at -20 °C for further experiments.
      *Note: The labeled DNA probes should be aliquoted before storing to avoid freeze-thaw cycles.*

B. Native polyacrylamide gel (5%) preparation
   1. Prepare 5% native polyacrylamide gel as follows (Table 1):

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**Table 1. Native polyacrylamide gel (5%) preparation**

| Reagent | Volumes of reagents for a 15 ml gel | Final concentration |
|---|---|---|
| 10x TBE | 750 µl | 0.5x |
| 30% acrylamide and bis-acrylamide solution (29:1) | 2.5 ml | 5% |
| TEMED | 15 µl | 0.001% (v/v) |
| 10% APS | 150 µl | 0.1% |
| Finally, add molecular biology-grade water to 15 ml | | |

2. Before using, pre-run the gels in 0.5x TBE buffer for 60 min (stable current and 120 V).

C. EMSA reaction

1. Each EMSA assay may require 7 reactions as illustrated in Table 2.
   *Note: It is recommended to include reaction #2 for a negative control using a non-binding protein. However, this reaction could be removed if you do not know any suitable non-binding protein. In addition, various concentrations (e.g., 50x, 100x, 200x, and 300x) of unlabeled DNA probes are included in this protocol. Nevertheless, it is also acceptable if only one concentration is included (as long as that amount of unlabeled DNA probes can compete with labeled DNA probes resulting in the abolishment of shifted-band).*

**Table 2. Typical designed reactions for an EMSA assay**

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|---|
| Labeled DNA probe | + | + | + | + | + | + | + |
| Unlabeled DNA probe | - | - | - | 50x | 100x | 200x | 300x |
| Water (for no-protein negative control) | + | - | - | - | - | - | - |
| Non-binding protein | - | + | - | - | - | - | - |
| Interested protein | - | - | + | + | + | + | + |

2. Freshly prepare the EMSA mixture as indicated in Table 3 (for 8 reactions) and distribute 17 µl of this pre-mixture to each micro-tube (for one reaction).
   *Note: It is better to prepare a pre-mixture containing all reagents listed in Table 3 for 8 reactions (to avoid the loss during handling). Subsequently, recombinant protein can be added to designed reactions.*

**Table 3. Ingredients of a pre-mixture (for 8 reactions)**

| Reagent | Volumes (for 8 reactions) | Final concentration |
|---|---|---|
| Tris 1 M pH 7.5 | 4 µl | 25 mM |
| NaCl 1 M | 12.8 µl | 80 mM |
| KCl 1 M | 5.6 µl | 35 mM |
| Dithiothreitol (DTT) 100 mM | 1.6 µl | 1 mM |
| Glycerol | 16 µl | 10% |
| $MgCl_2$ 100 mM | 8 µl | 5 mM |
| poly(dI.dC) | 8 µl | 10 ng/µl |
| NP-40 10% | 32 µl | 2% |
| Molecular biology-grade water | 48 µl | |

3. Add interested recombinant protein (1 µg/µl) as indicated in Table 4.
   *Note: In this protocol, it is recommended to include the reaction #2 which is negative control using a non-binding protein. However, as mentioned in Step C1, this reaction can be removed if you do not know any suitable non-binding protein.*

**Table 4. A detailed guide for adding the interested recombinant protein to each reaction (#1-#7)**

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|---|
| Water (for no-protein negative control) | 1 µl | - | - | - | - | - | - |
| Non-binding protein | - | 1 µl | - | - | - | - | - |
| Interested protein | - | - | 1 µl | 1 µl | 1 µl | 1 µl | 1 µl |

4. Next, add 1 µl of the unlabeled DNA probes (or molecular biology-grade water) (as shown in Table 5), gently mix up and let the reaction at room-temperature (25 °C) for 15 min.

**Table 5. A detailed guide for adding the unlabeled DNA probes to each reaction (#1-#7)**

| | #1 | #2 | #3 | #4 | #5 | #6 | #7 |
|---|---|---|---|---|---|---|---|
| Water | 1 µl | 1 µl | 1 µl | - | - | - | - |
| Unlabeled DNA probe | - | - | - | 50x | 100x | 200x | 300x |

5. Finally, add 1 µl of the labeled DNA probes (50 nM) to each reaction (#1-#7), gently mix up and let the reaction at room-temperature (25 °C) for 60 min.

D. Electrophoresis

1. Add 3.5 µl of 6x DNA Gel Loading Dye to each reaction, gently mix up and load an equal amount of each reaction in the gel which was pre-run above.
   *Note: If gel-well's capacity cannot handle all 23.5 µl, it is fine to load a lesser amount (e.g., 20 µl per well).*

2. Electrophoresis conditions: stable current and 120 V at room-temperature in 0.5x TBE buffer.
3. Running time: around 30 min (can stop running when you see the dye reaches 2/3 down length of the gel).

E. Transferring

1. During electrophoresis, cut the membrane to fit the gel. In this protocol, the Mini Trans-Blot® system (Bio-Rad, US) is used.
2. After electrophoresis, carefully transfer the gel onto a filter paper, foam pad, and the black-side of the gel holder cassette as demonstrated in Figure 1. Place the membrane (from Step E1) on the gel. Carefully remove the air-bubbles between the membrane and the gel (can use the Bio-Rad roller or can roll a measuring pipette on the membrane). Next, place one more filter paper and a foam pad on the membrane. Lock the gel holder cassette and transfer this to the buffer tank containing the electrode (Figure 1). Fill up the buffer tank with 0.5x TBE buffer. Next, cover the lid of the buffer tank and start the transferring.
3. Transferring conditions: stable current and 100 V at room-temperature in 0.5x TBE buffer. And the transferring time is 30 min.

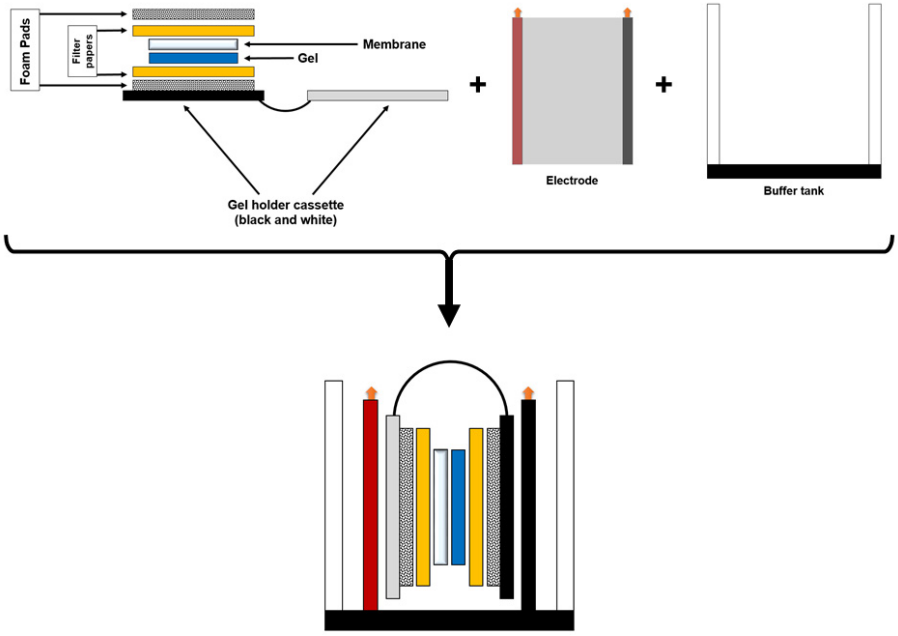


**Figure 1. The graphical demonstration for the transferring step**

F. Cross-linking

1. After transferring, carefully place the membrane on a holder (*e.g.*, aluminum foil). The gel-contacted surface should be faced up.
2. Gently put the holder containing the membrane to the UV-cross-linker. Next, close the UV-cross-linker door carefully. Cross-linking can be carried out in a UV-cross-linker with 254 nm bulbs (120 mJ/cm$^2$ for 1 min).

*Note: UV light is harmful for humans. Make sure that the UV light is completely turned off before open the cross-linker.*

3. Subsequently, the membrane can be used for detection or stored at room-temperature for a few days.

G. Detection

The Chemiluminescent Nucleic Acid Detection Module Kit (Thermo Fisher Scientific) is used and the detection step follows the company's instruction.

*Note: For the final detection step, a bio-imaging system equipped with a charge-coupled device (CCD)-camera or even conventional method using X-ray film can be used.*

## Data analysis

The obtained result can be exhibited as Figures 2 and 3. As shown in Figure 3, the band indicating the shifted probe was observed when the interested protein (an MYB transcription factor) binds to its targeted DNA. In the neighbor reaction, the unlabeled probe (300x) was added and these unlabeled DNA strongly competed with the labeled one (1x) resulting in the abolishment of the shifted band. Besides, there were no shifted probes in the negative control assay (the interested protein and non-targeted DNA).

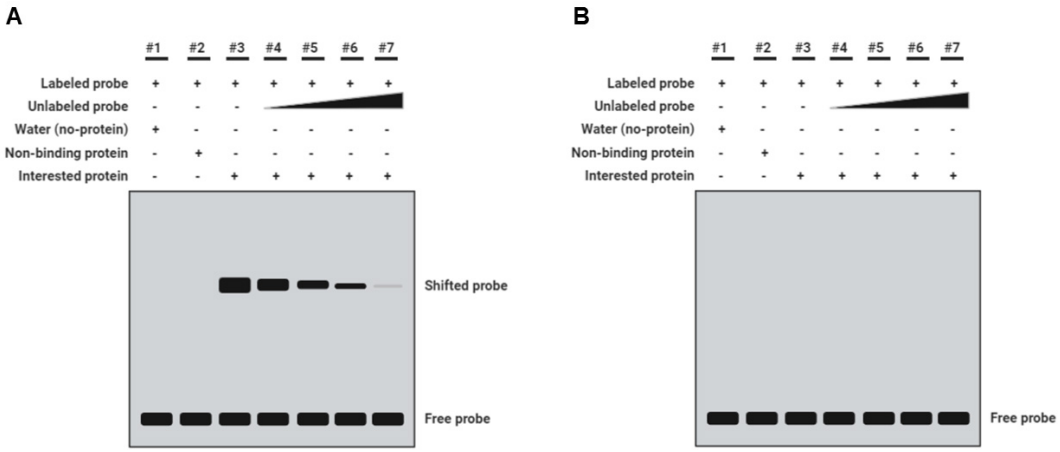


**Figure 2. Typical results of EMSA assays (graphical).** A. The interested protein and targeted DNA interact. B. There are no interactions between interested protein and DNA.

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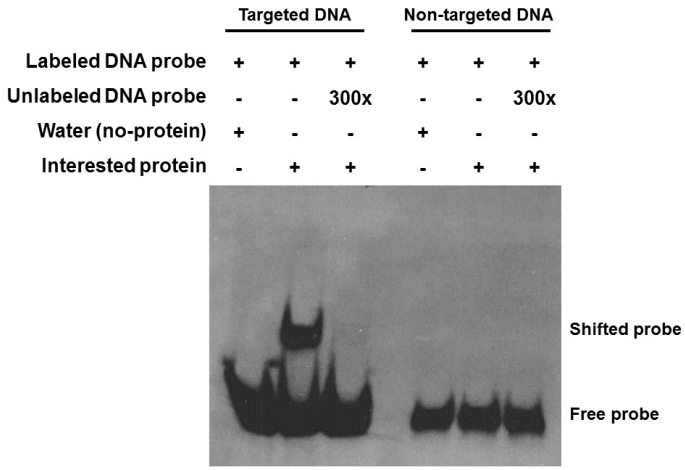


**Figure 3. An experimental result of EMSA assay followed the present protocol.** In this EMSA assay, the recombinant protein (a MYB transcription factor) and it's targeted as well as non-binding DNA probes were used.

## Recipes

1. 1 M and 100 mM DTT
   To prepare 10 ml of 1 M DTT
   a. Dissolve 1.545 g of DTT in around 5 ml of molecular biology-grade water
   b. Next, add more molecular biology-grade water to achieve 10 ml
   c. Mix 1 ml of 1 M DTT with 9 ml of molecular biology-grade water to achieve 10 ml of 100 mM DTT
   d. Aliquot 1 ml to each micro-tube and store at -20 °C
2. 10x TBE buffer
   To prepare 1 L of 10x TBE buffer
   a. Mix Tris base (108 g), boric acid (55 g), and EDTA (disodium salt) (9.3 g) with molecular biology-grade water (~500 ml)
   b. Next, add more molecular biology-grade water to achieve 1 L
   c. Autoclave for 20 min and this 10x TBE buffer can be stored at room-temperature

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