A detailed and radioisotope-free protocol for electrophoretic mobility shift assay (EMSA)

NGUYEN HOAI NGUYEN (nguyen.nhoai@ou.edu.vn)
Ho Chi Minh City Open University https://orcid.org/0000-0002-0647-9724

Method Article

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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. This protocol is to provide a detailed procedure for a safe EMSA assay (without using any radioisotope).

Introduction

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 Biotin and the shifted band can be visualized via chemiluminescence.

Reagents

1. Interested recombinant proteins.
2. Labeled and unlabeled (3'-end Biotin) oligo DNA probes (sense and anti-sense) (**see note 1**).
3. Tris pH 7.5
4. NaCl
5. KCl
6. DTT (Dithiothreitol) (Catalog number: 3483-12-3, Sigma-Aldrich, US)
7. Glycerol
8. $MgCl_2$
9. poly(dI.dC) (Catalog number: 20148E, Thermo Fisher Scientific, US)
10. NP-40 (Catalog number: 28324, Thermo Fisher Scientific, US)

11. 30% Acrylamide/Bis Solution, 29:1 (Catalog number: 1610156, Bio-Rad, US)

12. TEMED (N,N,N',N'-tetramethylethylenediamine) (Catalog number: 161-0801, Bio-Rad, US)

13. APS (ammonium persulfate) (Catalog number: A3678, Sigma-Aldrich, US)

14. Positively charged nylon membrane (GE Healthcare Amersham™ Hybond™-N+) (Catalog number: 45-000-927, Thermo Fisher Scientific, US)

15. 6x DNA Gel Loading Dye (Catalog number: R0611, Thermo Fisher Scientific, US)

16. 10x TBE buffer (**see recipe**)

17. Chemiluminescent Nucleic Acid Detection Module Kit (Catalog number: 89880, Thermo Fisher Scientific, US)

Equipment

1. 1.5 ml micro-tubes
2. Centrifuge
3. Heat-block
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
8. Bio-imaging system equipped with a charge-coupled device (CCD)-camera (e.g. ChemiDoc, Bio-Rad, US)

Procedure

1. Double-strand DNA probe preparation

- Mix the single-strand sense and antisense oligos (100 μM) (ratio 1:1) in a micro-tube (**see note 1**).

- Place the micro-tube in a heat-block at 95^{o}C for 5 min.

- Afterward, turn off the heat-block. Remove the mental rack containing the micro-tube and let all cool down at room-temperature (**see note 2**).

- Aliquot and add molecular biology-grade water to dilute the labeled DNA probes to 50 nM (1x) and unlabeled DNA probes to 2500 nM (50x), 5000 nM (100x), 10000 nM (200x), and 15000 nM (300x).

- Store at -20^{o}C for further experiments (**see note 3**).

2. Native polyacrylamide gel (5%) preparation

- Prepare 5% native polyacrylamide gel as follows:

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

3. EMSA reaction

- Each EMSA assay requires at least 7 reactions as illustrated in Table 1.

- Freshly prepare the EMSA mixture as indicated in Table 2 (for one reaction) (**see note 4**).

- Next, add 1 µl of the unlabeled DNA probes (as shown in Table 3), gently mix up and let the reaction at room-temperature for 15 min.

- 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 for 60 min.

4. Electrophoresis

- Add 3.5 µl of 6x DNA Gel Loading Dye to each reaction, gently mix up and load an equal amount of each reaction (**see note 5**) in the gel which was pre-run above.

- Electrophoresis conditions: stable current and 120 V at room-temperature in 0.5x TBE buffer.

- Running time: around 30 min (can stop running when you see the dye reaches 2/3 down length of the gel).

5. Transferring

- During electrophoresis, cut the membrane to fit the gel.

- Transferring conditions: stable current and 100 V at room-temperature in 0.5x TBE buffer.

- Transferring time: 30 min.

6. Cross-linking

- After transferring, carefully place the membrane on a holder (e.g. aluminum foil). The gel-contacted surface should be faced up.

- Cross-linking can be carried out in a UV-cross-linker with 254 nm bulbs (120mJ/cm^2 for 1 min).

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

7. Detection

- The Chemiluminescent Nucleic Acid Detection Module Kit (Thermo Fisher Scientific) can be applied for detection (**see note 6**).

Troubleshooting

- **Note 1:** In this protocol, the length of oligos was 40 bp. The biotin was only added to 3'-end of sense oligo. These oligos were ordered from a local company and it also provided a service to label oligo with biotin. If there is no such that service, the Pierce™ Biotin 3' End DNA Labeling Kit (Catalog number: 89818, Thermo Fisher Scientific, US) can be considered. In addition, it is better to include one more EMSA assay (for negative control) with a non-targeted DNA probe.

- **Note 2:** 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^{o}C.

- **Note 3:** The labeled DNA probes should be aliquoted before storing to avoid freeze-thaw cycles.

- **Note 4:** It is better to prepare a pre-mixture containing all reagents listed in Table 2 (excepting recombinant protein) for 8 reactions (to avoid the loss during handling) and distribute this pre-mixture equally to each reaction. Subsequently, recombinant protein can be added to designed reactions.

- **Note 5:** If gel-well's capacity cannot handle all 25 µl, it is fine to load a lesser amount (e.g. 20 µl) per each.

- **Note 6:** 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.

Time Taken

1 - 2 days

Anticipated Results

References

Acknowledgements

Figures

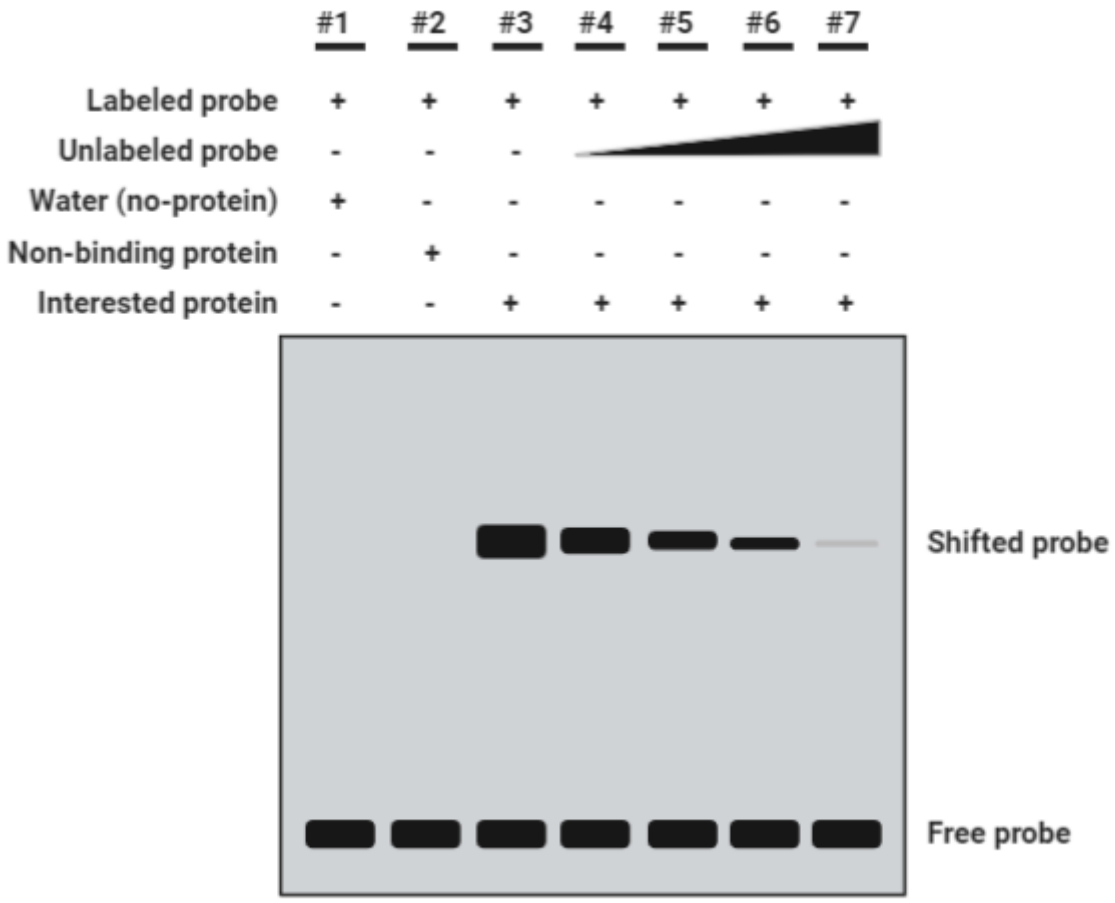

Figure 1

Figure 1. A typical result of an EMSA assay which indicates that the interested protein and targeted DNA interact.

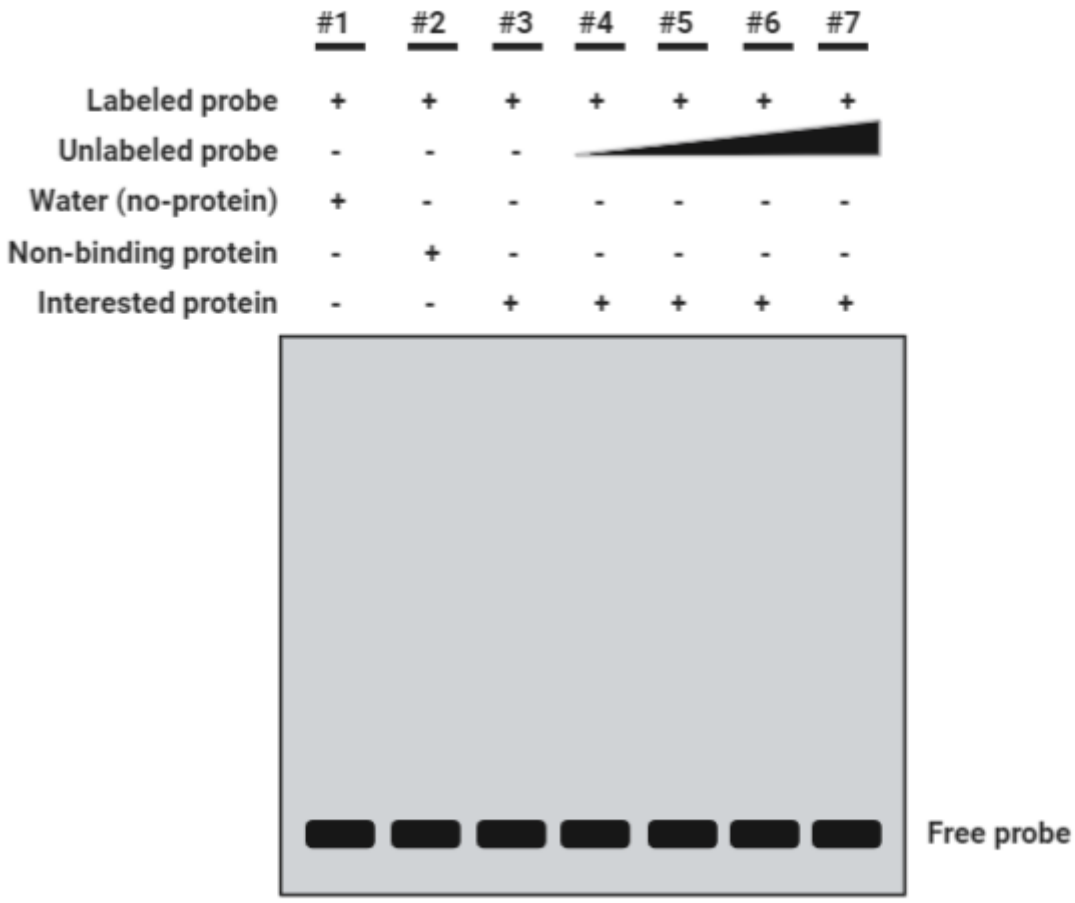

Figure 2

Figure 2. A typical result of an EMSA assay which indicates that there are no interactions between interested protein and DNA