Electrophoresis Mobility Shift Assay

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[Abstract] Protein (transcription factors and/or transcription cofactors)-binding to DNA is a critical event in regulation of transcription. Electrophoresis Mobility Shift Assay (EMSA), also known as gel shift assay, is a useful tool to detect protein- or protein complex-DNA/RNA interaction and to evaluate DNA binding specificity of transcription factors *in vitro*. Here we describe a simple method for EMSA with fluorescent dye-bound oligo DNA probes and recombinant protein expressed in bacterial cells. Using fluorescent dye instead of radioisotope enables easy handling and long-term storage of labelled-probes without reduction of detection sensitivity.

Materials and Reagents

1. Oligo DNA 5' end-labeled with IRDye 700 or IRDye 800 (sense strand) (Integrated DNA Technologies)
2. Non-labelled oligo DNA (both sense and antisense strands)
3. Non-labelled mutated oligo DNA (both sense and antisense strands)
4. Recombinant DNA-binding proteins expressed in *Escherichia coli (E. coli)* (10 ng/µl in protein storage buffer)
5. Sterile distilled water (SDW)
6. Odyssey Infrared EMSA kit (LI-COR, catalog number: 829-07910)
7. Poly(dI-dC) (Sigma-Aldrich, catalog number: 4929)
8. Tris
9. Boric acid
10. EDTA 2Na
11. NaCl
12. HCl
13. Acrylamide
14. N,N'-Methylene-bisacrylamide
15. Glycerol
16. Triton X-100

17. Phenylmethylsulfonyl fluoride (PMSF)
18. β-mercaptoethanol
19. Ammonium persulfate (APS)
20. N,N,N',N'-tetramethylethylenediamine (TEMED)
21. 10x TBE(see Recipes)
22. 4% native polyacrylamide gel (see Recipes)
23. Native-PAGE running buffer (see Recipes)
24. Protein storage buffer (see Recipes)

Equipment

1. Odyssey CLx Infrared Imaging System (LI-COR)
2. A set of devices for polyacrylamide gel electrophoresis
3. Power supply
4. Refrigerator or cold room
5. Heat block

Procedure

1. Suspend lyophilized oligo DNA with dH_2O and mix them as follows (final concentration is 50 μM each).
   Probe: labelled and complementary non-labelled oligo DNAs
   Competitor: non-labelled and complementary non-labelled oligo DNAs
   Mutated competitor: mutated non-labelled and complementary non-labelled oligo DNAs
2. Heat at 100 °C for 10 min on heat block for denature.
3. Turn off heat block and leave denatured DNAs on the block until room temperature.
4. Dilute probes to 50 nM with dH_2O.
5. Prepare 4% native polyacrylamide gel and 0.5x TBE.
6. Pre-electrophoresis for 30 min at 150 V at 4 °C.
7. Prepare reaction mixtures as follows during pre-electrophoresis:

	Negative Control	Probe	Competitor	Mutated competitor
Protein (10 ng/µl)	-	1µl	1µl	1µl
Probe (50 nM)	1 µl	1 µl	1 µl	1 µl
Competitor (50 µM)	-	-	1 µl	-
Mutated competitor (50 µM)	-	-	-	1 µl
10 x buffer (tube 1)	2 µl	2 µl	2 µl	2 µl
25 mM DTT/2.5% Tween-20 (tube 2)	2 µl	2 µl	2 µl	2 µl
1 µg/µl Poly (dIdC) (tube 3)	1 µl	1 µl	1 µl	1 µl
50% Glycerol (tube 5)	2µl	2µl	2µl	2µl
100 mM $MgCl_2$ (tube 8)	1µl	1µl	1µl	1µl
dH_2O	11 µl	10 µl	9 µl	9 µl

Note: Tube numbers indicate the vial numbers in Odyssey Infrared EMSA kit.

8. Place at room temperature for 20 min in dark.
   Note: Avoid light during reaction not to reduce signal intensity.
9. Add 2 µl of 10x Orange Dye (tube 10) to reaction mixture after reaction.
10. Wash gel wells with electrophoresis buffer (0.5x TBE) after pre-electrophoresis.
11. Load the samples on gel and run the gel at 150 V for 2.5 h at 4 °C in dark until the Orange Dye migrates to the bottom of the gel.
12. Remove gel from glass plate and place it directly on Odyssey.
13. Adjust focus offset of Odyssey to 1/2 of gel thickness and scan.
    Note: Carefully remove air bubbles between gel and Odyssey.
    Look at supplemental Figure 19 of Reference 1 as a representative EMSA result.

Recipes

1. 10x TBE
   Consisting of 890 mM Tris, 890 mM boric acid and 20 mM EDTA 2Na (pH 8.3)
   Mix:
   108 g of Tris base
   55 g of boric acid
   3.7 g of EDTA 2Na
   Add dH_2O to 1 L
   Autoclave and stored at room temperature.
2. 4% native polyacrylamide gel
   Consisting of 4% acrylamide, 0.5x TBE, 2.5% glycerol, 0.1% APS and 0.1% TEMED

Mix :

2.67 ml of 30% acrylamide (acrylamide: bisacrylamide = 29 :1)

1 ml of 10x TBE

1 ml of 50% glycerol

0.2 ml of 10% APS

20 µl of TEMED

Add dH_2O to 20 ml

3. Native-PAGE running buffer

   0.5x TBE

4. Protein storage buffer

   Consisting of 10 mM Tris-HCl (pH7.5), 150 mM NaCl, 0.1% Triton X-100, 1 mM PMSF, 0.05% β-mercaptoethanol and 50% glycerol

   Mix : 1 ml of 1 M Tris-HCl (pH 7.5)

   3 ml of 5 M NaCl

   1 ml of 10% Triton X-100

   1 ml of 100 mM PMSF

   50 µl of β-mercaptoethanol

   50 g of glycerol

   Add dH_2O to 100 ml

References

1. Nakata, M., Mitsuda, N., Herde, M., Koo, A. J., Moreno, J. E., Suzuki, K., Howe, G. A. and Ohme-Takagi, M. (2013). A bHLH-type transcription factor, ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR/JA-ASSOCIATED MYC2-LIKE1, acts as a repressor to negatively regulate jasmonate signaling in *Arabidopsis*. *Plant Cell* 25(5): 1641-1656.

