A General EMSA (Gel-shift) Protocol

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[Abstract] An electrophoretic mobility shift assay (EMSA), also referred to as mobility shift electrophoresis, a gel shift assay, gel mobility shift assay, band shift assay, or gel retardation assay, is a common technique used to study protein-DNA or protein-RNA interactions. The control lane (the DNA/RNA probe without protein present) will contain a single band corresponding to the unbound DNA or RNA fragment. If the protein is capable of binding to the fragment, the lane with protein present will contain another band that represents the larger, less mobile complex of nucleic acid probe bound to the protein, which is 'shifted' up on the gel (since it has moved more slowly). Here, a protocol to carry out an EMSA assay is described.

Materials and Reagents

1. DTT (Promega Corporation, catalog number: V3151)
2. Poly-dIdC (Sigma-Aldrich, catalog number: P4929-10UN)
3. ^{32}P-labeled probe
   Note: Oligo DNA probe can be synthesized ordered from IDT, a DNA synthesis company, then labeled by yourself.
4. BSA (Sigma-Aldrich, catalog number: 05470-5G)
5. General chemicals (Sigma-Aldrich)
6. 5x binding buffer (see Recipes)
7. 10x TBE buffer (see Recipes)

Equipment

1. Plates
2. Spacers
3. Clamps
4. Saran wrap
5. Whatman paper (GE Healthcare)

Procedure

1. Pour protein polyacrylamide gel.
   a. Assemble plates, spacers, and clamps. Seal with 1% agarose to prevent leaks.
   b. Pour 5% polyacrylamide gel.

Plate size	Large	Medium
H_2O	78 ml	39 ml
10x TBE	5 ml	2.5 ml
30% acrylamide stock (19:1)	16.6 ml	8.4 ml
10% APS	1,000 µl	500 µl

   Mix well while minimizing bubble formation. Add 100 µl/50 µl TEMED. Mix and pour, add combs. Gel will take ~10 min to polymerize. After polymerization, gel can be wrapped in saran wrap and stored at 4 °C.
2. Prepare 5x binding buffer.
3. Set binding reaction:
   1 µl of poly-dIdC (1 µg/µl in TE)
   2 µl of 5x binding buffer
   1 µl of labeled probe
   1 µl cold competitor - unlabeled DNA fragments containing the binding sequences (if needed)
   0.1 µl 100x BSA
   X µl nuclear extract (5 µg protein total)
   Add H_2O to 10 µl final volume
   Incubate for 30 min at room temp. Add antibody for supershift (if needed). Incubate additional 30 min at room temp.
4. While binding reaction is incubating, run the polyacrylamide gel without any sample at 150 V, 30 min, using 0.5x TBE as the running buffer. Then run samples on the polyacrylamide gel for ~2 h at 150 V.
5. Dry the gel (optional).
   Transfer gel to Whatman paper. Cover top of gel with saran wrap and dry at 80 °C in vacuum dryer for 1-2 h.
6. Expose the gel.
   Place gel in cassette with reflection screen. Add film and place in -80 °C freezer.

Recipes

1. 5x binding buffer

Composition	Recipe for 10 ml
50 mM Tris HCl (pH 8.0)	0.5 ml of 1 M Tris HCl (pH 8.0)
750 mM KCl	3 ml of 2.5 M KCl
2.5 mM EDTA	50 µl of 0.5 M EDTA (pH 8.0)
0.5% Triton-X 100	50 µl Triton-X 100
62.5 % glycerol (v/v)	7.87 g glycerol
1 mM DTT	add DTT fresh before use

2. 10x TBE buffer (1 L)
   106 g of Tris base
   55 g of boric acid
   40 ml of 0.5 M EDTA (pH 8.0)

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References

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