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CHAPTER

ELECTRO- PHORETIC MOBILITY SHIFT ASSAY

Chapter Seven: Electrophoretic mobility shift assay (EMSA)

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Electrophoretic mobility shift assay overview

The Electrophoretic Mobility Shift Assay (EMSA) also referred to as the gel retardation assay or gel shift assay, is a common technique used to characterize protein:DNA/RNA interactions. Gel shift assays are often performed concurrently with DNase footprinting, ChIP and primer extension assays. EMSA/gel assay is based on the observation that complexes of protein and DNA/RNA migrate through a nondenaturing polyacrylamide gel more slowly than free DNA/RNA fragments or double-stranded oligonucleotides. The gel shift assay is performed by incubating a purified protein, or a complex mixture of proteins with a ³²P biotinylated labeled or hapten end-labeled DNA/RNA fragment containing the putative protein binding site and nonspecific DNA competitors usually polyanion polymers such as poly(dI-dC) or poly(dG-dC). These repetitive polymers provide an excess of nonspecific sites to adsorb proteins in crude lysates that will bind to any DNA or RNA sequence. The complex is then analyzed on a nondenaturing polyacrylamide gel or TAE agarose gel. The ability to resolve protein:DNA/RNA complexes depends largely upon the stability of the complex during its migration into the gel. Sequence-specific interactions are transient and are stabilized by the relatively low ionic strength of the electrophoresis buffer used.

The specificity of an observed DNA/RNA binding reaction can be evaluated using competition assays in which an excess of unlabeled probe is added together with the labeled probe. Specific DNA or RNA binding will be eliminated by a reasonable excess (10–100 molar excess) of unlabeled probe (unlabeled specific competitor).

In addition to a labeled DNA fragment, specific antibodies can be added to the gel shift reaction. Addition of a specific antibody to a binding reaction can have one of several effects. If the protein recognized by the antibody is not involved in complex formation, addition of the antibody should have no effect. If the protein that forms the complex is recognized by the antibody, the antibody can either block complex formation, or it can form an antibody-protein-DNA complex, resulting in a further reduction in the mobility of the protein-DNA complex (supershift).



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EMSA assay formats (Figure 12)

Target proteins may be obtained from crude cellular extracts or cell-free expression systems, or may be purified from *E. coli* or mammalian expression systems. Cellular extracts are easy to prepare and allow protein:DNA/RNA interactions to occur in a true cellular environment in which other

proteins may play a critical role. DNA/RNA binding proteins generated by cell-free expression systems or by purification from other expression systems offer an excellent format for the confirmation of data obtained from cellular extracts, and for characterization of the context of the protein:DNA interactions.

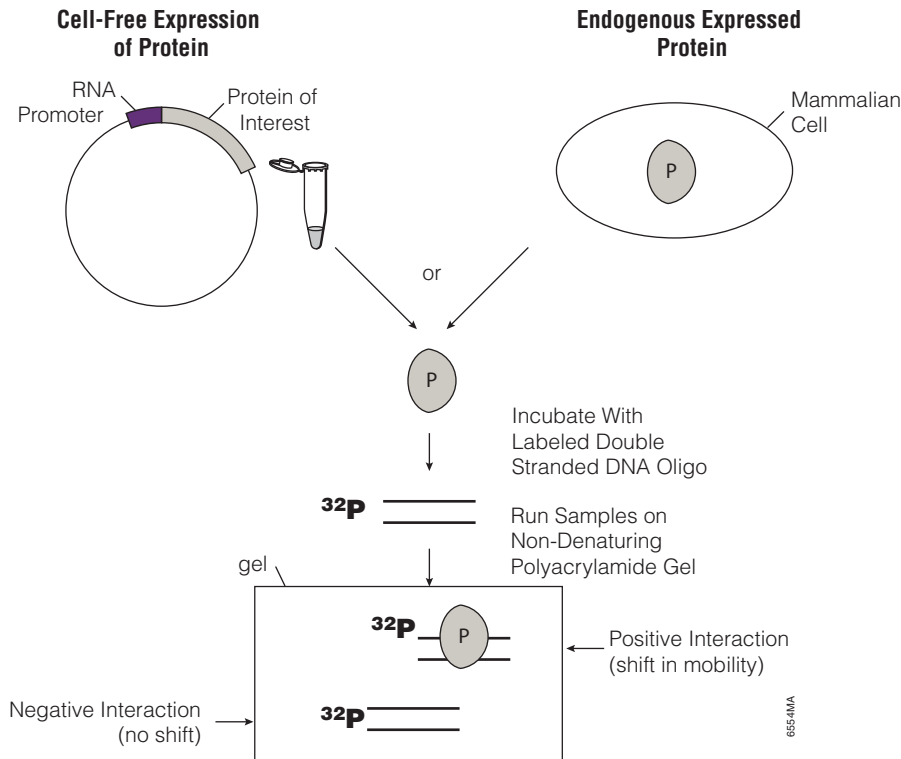


Figure 12. Overview of EMSA. The target protein is expressed in mammalian cells and a whole-cell extract is prepared. An alternate method relies on expressing the protein using mammalian-based, cell-free expression systems. In either case an aliquot containing the protein is incubated with the DNA sequence that has been labeled using either radioactive or non-radioactive methods and the DNA:protein complex is allowed to form. In order to maintain the protein:DNA complex, the reaction is run on a non-denaturing polyacrylamide gel. After electrophoresis, the experimental reaction is compared to a control reaction that contains only the labeled DNA to determine whether a protein:DNA interaction has occurred.

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When to use EMSA

EMSA can be used in conjunction with other techniques to characterize the transcriptional regulation mechanism of various genes. It can also be used to further characterize known transcriptional events to determine the effect of various stimuli or other upstream proteins that are required for successful interactions.

Primary reagent requirements for EMSA

- Neutral non-denaturing polyacrylamide gel
- Labeled and unlabeled DNA/RNA fragment containing putative binding site
- Target protein (from cellular extract, expressed in cell-free system or purified from mammalian or *E. coli* expression systems)
- Poly (dI-dC) or other nucleic acids to lower non-specific background

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