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(A CONSTITUENT PG COLLEGE OF UNIVERSITY OF ALLAHABAD)

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Molecular Biology and Molecular techniques

Unit (V): Topic: Protein- Nucleic acid interaction analysis

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Protein- Nucleic acid Interaction analysis

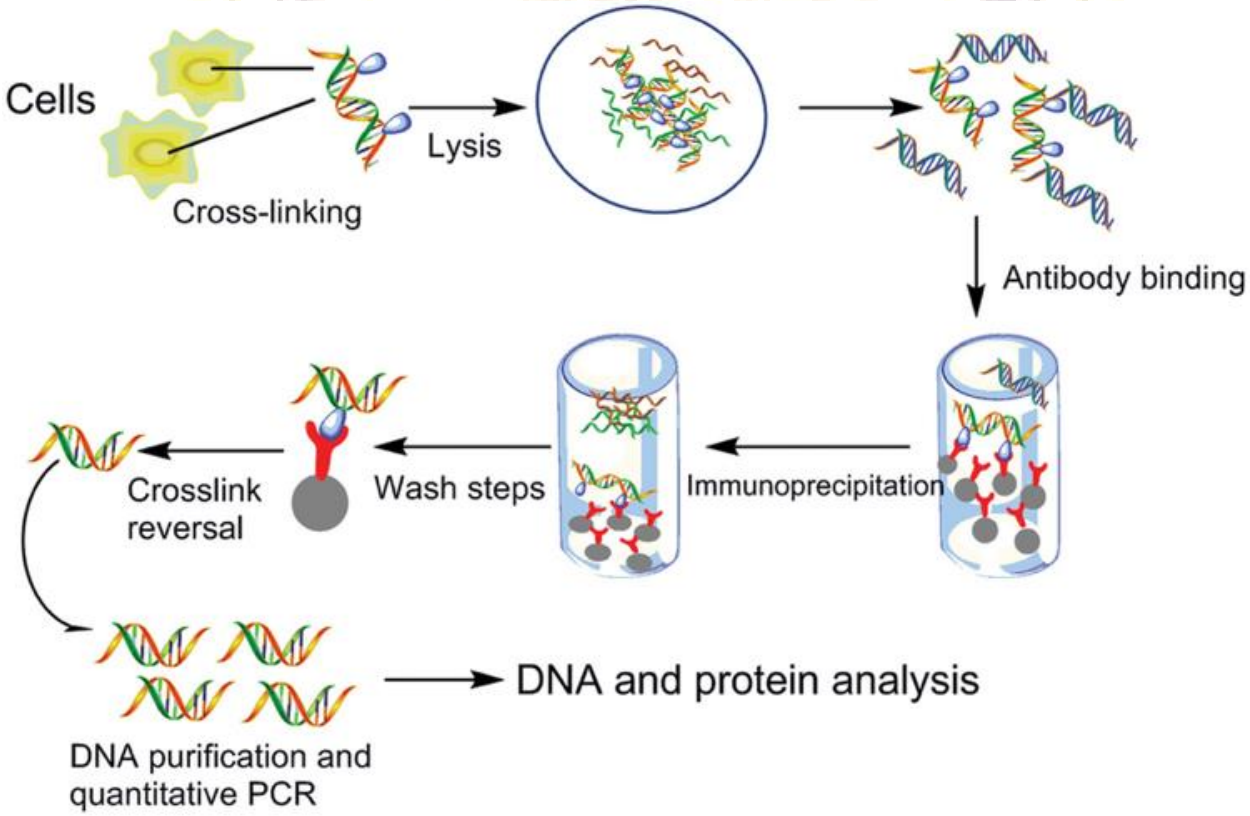
Two major techniques for detection of Protein-Nucleic acid interaction are:

1) **Chromatin immunoprecipitation (ChIP)**
2) **Electrophoretic mobility shift assay (EMSA)**

1) Chromatin immunoprecipitation (ChIP)

What Is Chromatin Immunoprecipitation?

Not only do proteins interact with one another, they can also interact with DNA. Chromatin immunoprecipitation (ChIP) is a technique that determines whether a protein of interest interacts with a specific DNA sequence. This technique is often used to study the repertoire of sites on DNA that are bound by particular transcription factors or by histone proteins, and to look at the precise genomic locations of various histone modifications (including acetylation, phosphorylation, or methylation).



How Does ChIP Work?

ChIP can be used to examine the presence of protein-DNA interaction at steady state, or to quantify changes in interaction at specific phases of the cell cycle, or following a treatment of interest. Protein and associated chromatin are temporarily cross-linked in live cells or tissues (using formaldehyde or

UV) and sheared using enzymatic digestion or sonication to yield ~300-1000 bp fragments of DNA. The protein of interest, along with any associated DNA fragments, is immunoprecipitated from the cell debris using a specific antibody. The cross-link is then reversed and DNA fragments are purified. The amount of eluted DNA can be assessed through quantitative real-time PCR (qRT-PCR) using primers flanking the genomic locus of interest. DNA amplification is an indication of enrichment in binding of the protein of interest.

Modified ChIP Techniques

DNA fragments purified by ChIP can be utilized for a number of downstream analysis techniques. Furthermore, the basic ChIP protocol described above can be modified to answer additional biological questions.

ChIP-on-chip: Genome-wide analysis of protein binding sites using microarray analysis of purified DNA fragments.

ChIP-Seq: Genome-wide analysis of protein binding sites using deep sequencing of purified DNA fragments.

Native ChIP: Omits the cross-linking step and uses micrococcal nuclease digestion to cut DNA at histone linkers to examine the DNA target of histone modifying proteins.

ChIP-exo: Addition of an exonuclease digestion step to obtain increased resolution of protein binding sites, up to a single base pair.

ChIA-PET (chromatin interaction analysis by paired-end tag sequencing): A technique that combines the principles of ChIP with chromosome conformation capture (3C) to detect long-range chromatin interactions mediated via a protein of interest.

iChIP (indexing-first chromatin immunoprecipitation): A high-sensitivity technique that reduces the number of cells required for a ChIP experiment by initially barcoding total cellular chromatin.

enChIP (engineered DNA-binding molecule-mediated chromatin immunoprecipitation): A technique which employs the CRISPR/Cas9 system to target specific genomic regions. A guide RNA complementary to the desired genomic region is expressed in combination with a tagged, enzymatically inactive Cas9 protein. ChIP is then performed using an antibody against the modified Cas9. This technique can help evaluate cis- and trans-interacting chromosomal looping events.

RIP-Chip/RIP-Seq: Similar techniques used to analyze protein-RNA interactions.

Limitations of ChIP

As with all molecular biology techniques, ChIP is not without its own set of limitations. ChIP assays often yield low signals as compared with controls, leading to inconclusive data. The assay is limited to a resolution relative to the size of the DNA fragments generated following shearing, which makes it

difficult to determine the exact binding site of a protein. While ChIP will infer the presence of a protein at a given genomic locus, it cannot determine functional significance of the protein's binding at that DNA region. Cross-linking may additionally include proteins that transiently interact with DNA or DNA-binding proteins and have no functional significance. Similarly, DNA interactions of proteins with short residence time (as little as several seconds for some transcription factors) may not be fully captured. Additionally, interacting proteins may mask the epitope of the protein of interest. Finally, the ChIP technique is extremely dependent on the quality and specificity of the antibody employed and may not discriminate between different DNA-binding protein isoforms.

2) Electrophoretic Mobility Shift Assay (EMSA) Based Methods (Also known as gel shift assay or Band shift assay or Gel mobility shift or Gel retardation assay)

The interaction of proteins with DNA is central to the control of many cellular processes including DNA replication, recombination and repair, transcription, and viral assembly. One important technique for studying gene regulation and determining protein–DNA interactions is the electrophoretic mobility shift assay (EMSA). An advantage of studying protein–DNA interactions by an electrophoretic assay is the ability to resolve complexes of different stoichiometry or conformation. Another major advantage is that the source of the DNA-binding protein may be a crude nuclear or whole cell extract, in vitro transcription product or a purified preparation. EMSA can be used qualitatively to identify sequence-specific, DNA-binding proteins (such as transcription factors) in crude lysates and, in conjunction with mutagenesis, to identify the important binding sequences within the upstream regulatory region of a given gene. EMSA can also be utilized quantitatively to measure thermodynamic and kinetic parameters.

EMSA is extensively used to analyze nucleic acid–protein interactions. EMSA is based on the principle that DNA–protein complexes are larger and move slowly when subjected to nondenaturing polyacrylamide gel electrophoresis (PAGE), compared to the unbound (free) DNA probe. Since the rate of DNA migration is shifted or retarded when bound to protein, the assay is also referred to as a gel shift or gel retardation assay. DNA probes used in EMSA are typically double-stranded oligonucleotides of 20–25 bp containing a response element and can be radio-, fluoro-, or hapten-labeled. The DNA and crude nuclear extract or recombinant TF are incubated together in a binding reaction and separated by PAGE. A supershift assay can be performed to specifically assert

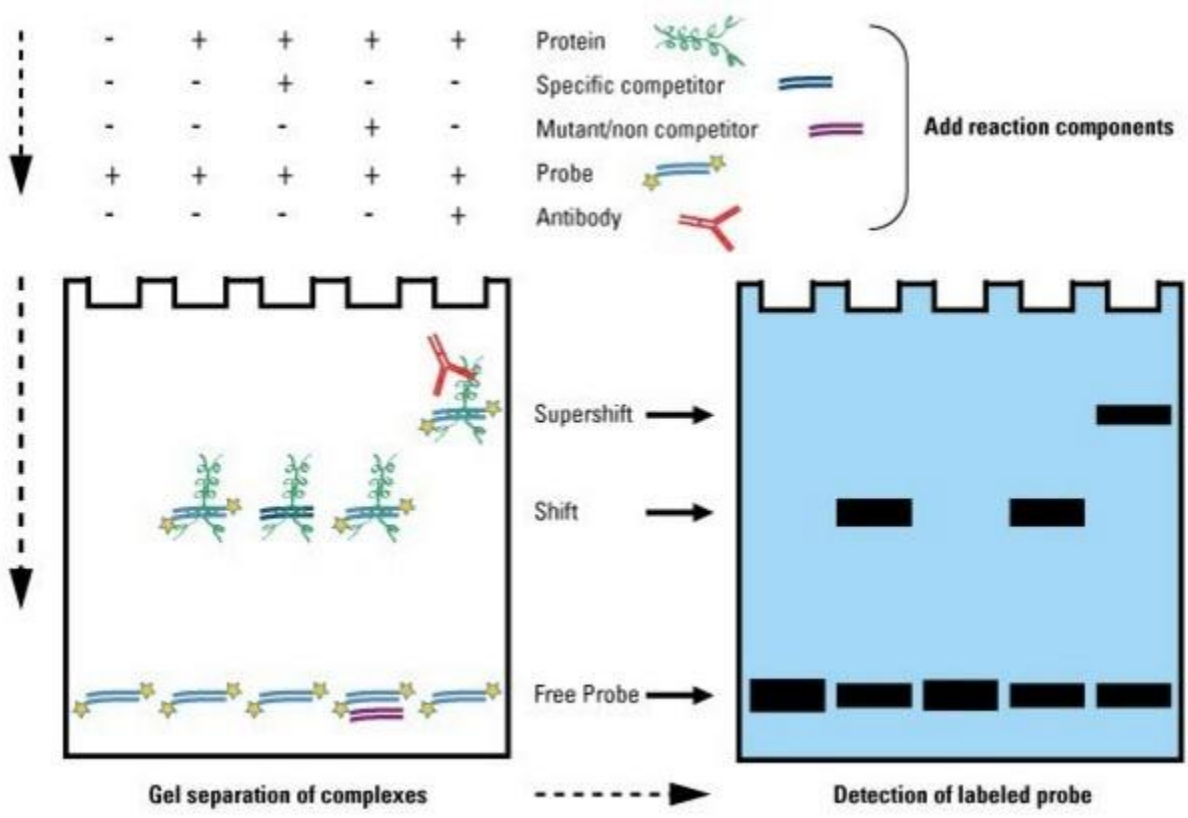
the DNA–protein interactions by using an antibody specific to the TF of interest. However, when the binding factor is unknown, two-dimensional-EMSA (2D-EMSA) can be used to identify sequence-specific DNA-binding proteins in crude nuclear extracts. 2D-EMSA utilizes the resolving power of SDS-PAGE to enable identification of proteins that, in a prior EMSA step, have altered mobility as a consequence of binding a probe. After purifying the protein band (spot) from the gel, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is used to identify the protein. This approach has been successfully employed for a bacterial lysate (Stead & McDowall, 2007), but the technique is challenging in cases of low-abundance TFs in a complex nuclear extract.

Introduction to the EMSA (gel shift) technique

The EMSA technique is based on the observation that protein–DNA complexes migrate more slowly than free linear DNA fragments when subjected to non-denaturing polyacrylamide or agarose gel electrophoresis. Because the rate of DNA migration is shifted or retarded when bound to protein, the assay is also referred to as a gel shift or gel retardation assay.

The ability to resolve protein–DNA complexes depends largely upon the stability of the complex during each step of the procedure. During electrophoresis, the protein–DNA complexes are quickly resolved from free DNA, providing a "snapshot" of the equilibrium between bound and free DNA in the original sample. The gel matrix provides a "caging" effect that helps to stabilize the interaction complexes: even if the components of the interaction complex dissociate, their localized concentrations remain high, promoting prompt reassociation. Additionally, the relatively low ionic strength of the electrophoresis buffer helps to stabilize transient interactions, permitting even labile complexes to be resolved and analyzed by this method.

Protein–DNA complexes formed on linear DNA fragments result in the characteristic retarded mobility in the gel. However, if circular DNA is used (e.g., mini-circles of 200–400 bp), the protein–DNA complex may actually migrate faster than the free DNA, similar to what is observed when supercoiled DNA is compared to nicked or linear plasmid DNA during electrophoresis. Gel shift assays are also good for resolving altered or bent DNA conformations that result from the binding of certain protein factors. Gel shift assays need not be limited to protein–DNA interactions. Protein–RNA and protein–peptide interactions have also been studied using the same electrophoretic principle.



Overview of the gel shift assay method. The gel shift assay consists of three key steps: (1) binding reactions, (2) electrophoresis, (3) probe detection. The order of component addition for the binding reaction is often critical. Completed binding reactions are best electrophoresed immediately to preserve potentially labile complexes for detection. This idealized example shows complete elimination of the protein–probe complex with the addition of a specific competitor or protein-specific antibody. However, only a reduction in intensity is observed rather than the complete elimination of bands.