Swayam Course - Analytical Techniques

Week 14, Tutorial 36 - Techniques for studying Nucleic Acid and Protein Interaction

Content Writer - Dr. Ruby Dhar, DBT-Biocare Fellow, Department of Biochemistry, All India Institute of Medical Sciences, New Delhi

Introduction

The characterization of protein–nucleic acid interactions is necessary for the study of a wide variety of biological processes. Protein: nucleic acid interactions play a major role in coordinating cellular functions, and this is especially important in regulating gene expression. For example, during the processes of replication, transcription, and translation, a whole host of proteins and enzymes bind to DNA or to RNA, and are integrally involved in copying the DNA, transcribing it into RNA, or translating the mRNA into protein. Crystallographic studies in the 1950s and early 1960s definitively established that proteins and nucleic acids do form specific and homogeneous three-dimensional structures, and by the late 1960s the principles governing the folding of these entities into their equilibrium forms were basically understood. The structural specificity of protein folding—meaning that each amino acid residue ends up in a specific position within the folded macromolecule—results from the requirement that the connectivity of the amino acid residues (held together by the largely polar polypeptide backbone) must be maintained. Various techniques have been developed to study nucleic acid and protein interaction. Microscopy was the initial method of choice and then X-Ray crystallography played a vital role and subsequently introduction of Gel retardation technique.

The crowning glory of 2009 was, of course, the award of the Nobel prize to Ada Yonath, Tom Steitz and Venki Ramakrishnan for the structure of the ribosome, the ultimate nucleic acid-protein machine whose structure has been determined.

In this module we will be discussing various techniques to study DNA-protein interaction.

OBJECTIVES

1.Introduction

- **1.1 What are DNA binding protein and their function**
- **1.2 What are RNA binding protein and their function**
- **1.3 Types of nucleic acid –protein interaction**

2. Microscopy to study nucleic acid-protein interaction

- **2.1 Electron microscopy and other types of microscopy**

3. Dnase footprinting assay

4. Gel shift assay /EMSA

5. Pull down assay

6. Reporter assay

7. Chromatin immunoprecipitation (ChIP)

8. SELEX (Systematic evolution of ligands by exponential enrichment)

9. Yeast 1 hybrid system

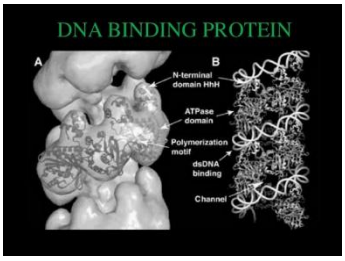
10. Summary

1. INTRODUCTION:

1.1 What are DNA-binding proteins?

- DNA-binding proteins control various cellular processes such as recombination, replication transcription , signaling , development and cell cycle control

DNA-binding proteins(DBPs), such as transcription factors, constitute about 10% of the protein-coding genes in eukaryotic genomes and play very important role in the regulation of chromatin structure and gene expression by binding to short stretches of DNA.Approximately every higher plant or vertebrate genome harbors over 2000 of these DBP genes.



Our knowledge on DNA-protein interaction is limited and in this module we discuss the various technologies to understand the interaction between DNA and Nucleic acid

1.2 WHAT ARE RNA BINDING PROTEINS:

- RNA-binding proteins are proteins that bind to ribonucleic acid (RNA) molecules

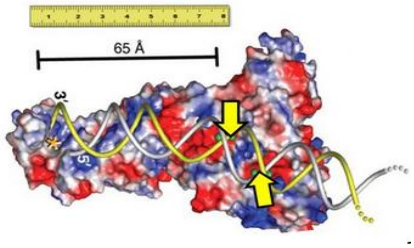
Generally found in the cytoplasm and nucleus, and are important in forming ribonucleoproteins (RNPs)

A type of RNP, hnRNPs (heteronuclear proteins), are important in splicing of mRNA (messenger RNA), polyadenylation, stabilization, localization and translation.

Proteins interact with RNA through electrostatic interactions, hydrogen bonding, hydrophobic interactions and base stacking in a manner similar to protein–DNA interactions. Protein–RNA interactions are also significantly influenced by the tertiary structure on the RNA molecules.Thus, in assays to identify protein–RNA interactions, both the RNA and protein(s) must be correctly folded to allow proper binding. RNA

is very susceptible to degradation, so special care must be taken not to introduce RNases into the reaction.

Example : DICER



Dicer is a RNA binding protein which binds to long dsRNA or hairpin RNA and cleaves to 21-25nt fragments

1.3 The Interaction between Nucleic acid and protein can be specific or nonspecific

SPECIFIC INTERACTION

The sequence of nucleotides directly affects the interaction outcome. Control transcription in prokaryotes and eukaryotes. This is mediated by hydrogen bonding, ionic interactions and Van de waal, forces.

NON-SPECIFIC INTERACTION

The binding interactions between protein and nucleic acid are not dependent on any specific sequence of nucleotides. Histone(protein)-DNA interactions are an example of such interactions and they occur between functional groups on the protein and sugar-phosphate backbone of DNA.

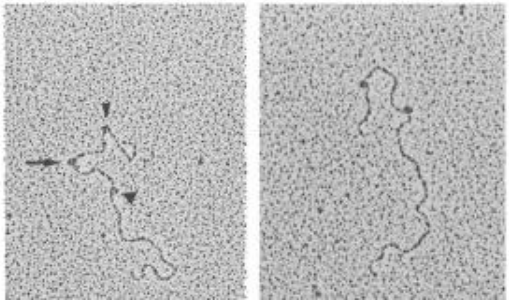
2 . Microscopy to study nucleic acid-protein interaction

2.1 Electron microscopy and other types of microscopy

During the late 19th century, scientists microscopically observed the association of proteins with DNA strands.

The most successful method of visualising nucleic acids (DNA and RNA molecules, DNA-RNA hybrids) was first reported by Kleinschmidt and Zahn (1959).

Researchers have used a variety of procedures to demonstrate that proteins interact with DNA and RNA to influence the structure and function of the corresponding nucleic acid. Protein-nucleic acid interactions therefore play a crucial role in central biological processes, ranging from the mechanism of Replication, transcription and recombination to enzymatic events utilizing nucleic acids as substrates.



Electron Microscopy of RNA polymerase II

and Estrogen receptor at a 5'DNA fragment of vitellogenin B2

There are three ways to study DNA Protein interaction using EM:

- The first method , cytochrome-c spreading, allows DNA to be visualized after it is adsorbed to a monolayer of unfolded and partially denatured cytochrome-c protein. Cytochrome c increases the diameter of the nucleic acid molecules to 15-20 nm. After spreading, the nucleic acid-protein film is adsorbed to a support membrane on a grid, contrasted by shadowing with heavy metals and observed in the EM.
- A second very general method simply consists of the direct adsorption of DNA-protein complexes onto a thin carbon film. The film can be activated in a variety of ways to increase the efficiency of adsorption. This method has the great advantage that there is generally very much less disruptive force applied to the complexes during adsorption and drying compared to the cytochrome-c procedure.
- The third method is an extension of either of the above methods to allow proteins to be specifically labeled with antibodies conjugated to gold particles. This method has the obvious advantage that it allows the unambiguous identification of the proteins involved in a DNA complex. Further more, if the protein of interest is too small for direct visualization, the gold spheres show approximate positions.

Other Microscopic techniques:

Microscopic techniques include optical, fluorescence, electron, and atomic force microscopy (AFM), the latter two providing the highest spatial resolution. Where the latter three resolve dynamic interactions, electron microscopy is limited to static observations.

AFM is arguably the most versatile microscopic method because It offers sub-nanometer resolution, images samples in liquids, and probes intermolecular forces between single molecules.

Structure determination using X-ray crystallography has been used to give a highly detailed atomic view of protein–DNA interactions.

3.DNASe FOOTPRINTING

DNA footprinting is a method of investigating the sequence specificity of *DNA*-binding proteins in vitro.

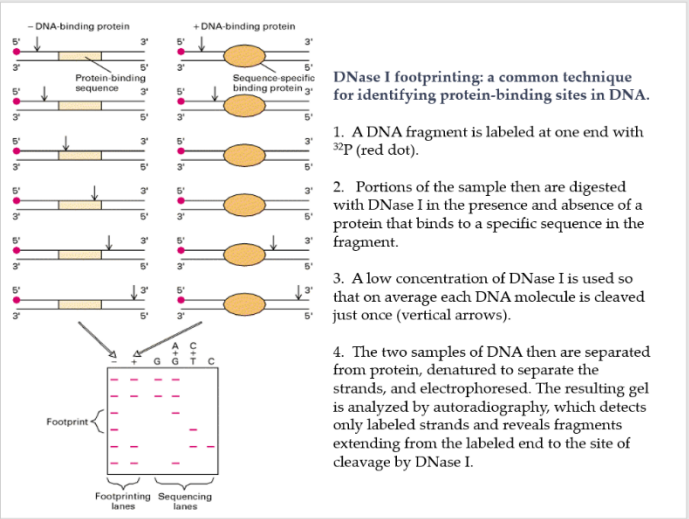
First established **by Galas and Schmitz in 1978**, it is one of the earlier techniques used to study these interactions

The basis of this technique is that DNA bound proteins protect DNA from external agents that are known to cleave or modify DNA. A protein under investigation is added to pieces of DNA containing putative protein-binding sites.

The DNA is treated with a cleaving or modifying agent and the products are run on a polyacrylamide gel.

When compared to a control DNA without added protein, the pattern of the test DNA would show gaps or 'footprints' where the protein of interest has bound to the DNA.

This region can then be analyzed and determined as a potential protein binding site.



SCHEMATIC DIAGRAM SHOWING DNAse FOOTPRINTING PROCESS:

4.Gel Shift assay /Electrophoresis mobility shift assay(EMSA)

- The gel electrophoresis mobility shift assay (EMSA) is used to detect protein complexes with nucleic acids.
- It is the core technology underlying a wide range of qualitative and quantitative analyses for the characterization of interacting systems.
- EMSA has been used widely in the study of sequence-specific DNA-binding proteins such as transcription 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.

- The assay is based on the observation that complexes of protein and DNA migrate through a non-denaturing polyacrylamide gel more slowly than free DNA fragments or double-stranded oligonucleotides.

- In the classical assay, solutions of protein and nucleic acid are combined and the resulting mixtures are subjected to electrophoresis under native conditions through polyacrylamide or agarose gel.
- After electrophoresis, the distribution of species containing nucleic acid is determined, usually by autoradiography of ^{32}P-labeled nucleic acid or using nonradioactive methods like oligos labelled with haptens (e.g., biotin and digoxigenin) or fluorescent dyes.

SELECTION OF PROBES FOR EMSA:

- Short nucleic acids are easily synthesized and inexpensive to purchase.
- The small number of non-specific protein binding sites in a small DNA or RNA can be advantageous

  when the binding protein has low sequence-specificity.

  On the other hand, all binding sites on a short nucleic acid are close to the

  molecular ends. This can result in aberrant binding due to structural and electrostatic end-effects.
- Longer templates avoid these limitations but contain more non-specific binding sites,

  migrate more slowly (requiring longer electrophoresis times) and generally give a smaller mobility-shift on protein binding.

Binding conditions

Protein-nucleic acid interactions are sensitive to mono- and divalent salt concentrations and Ph.

Commonly used buffers are include Tris based, HEPES, MOPS,

LABELLING PROBES AND DETECTION METHODS FOR EMSA:

Radioactive method:

DNA probes have been radiolabeled with ^{32}P by incorporating an [γ-^{32}P]dNTP during a 3' fill-in reaction using Klenow fragment or by 5' end labeling using [γ-^{32}P]ATP and T4 polynucleotide kinase. Following electrophoresis, the gel is exposed to X-ray film to document the results.

Non-radioactive method :

Many labs have moved to alternative EMSA detection systems due to the expense and regulatory concerns associated with radioactivity.

The only additional step required for this method is to transfer the separated protein and DNA samples onto an appropriate membrane support.

dNTPs can be modified with haptens (e.g., biotin and digoxigenin) or fluorescent dyes

Hapten-modified DNA probes can be visualized via secondary detection reagents such as streptavidin or anti-DIG antibodies in systems with enzymatic substrates similar to those used for western blotting.

ELECTROPHORESIS CONDITIONS:

The resolution of complexes depends on their stability during electrophoresis.

The composition and concentration of gel and running buffer components can be adjusted to optimize the stability of complexes.

The most popular buffers are variants of Tris-borate-EDTA, Tris-acetate-EDTA or Tris-glycine

Stabilizers such as glycerol or ethylene glycol can be included in gel- and running buffers to enhance the stability of complexes.

Although both polyacrylamide and agarose gels have been used for EMSA, polyacrylamide gels offer better electrophoretic resolution for protein-DNA and protein-RNA complexes

One effective optimization strategy is to start with a relatively low concentration gel (e.g., 5% w/v acrylamide) and to increase this concentration systematically until any improvement in complex stability is balanced by loss of electrophoretic resolution and/or the onset of impractically long electrophoresis times.

Nonspecific and specific competitors

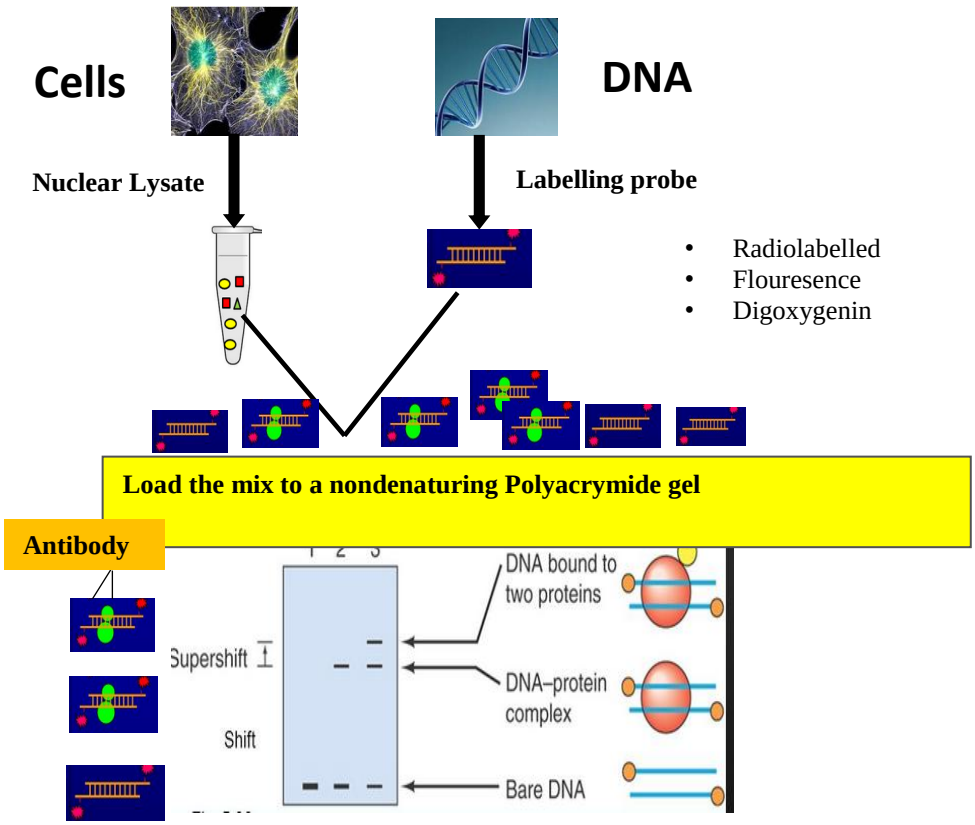
- A nonspecific competitor is any irrelevant, unlabeled nucleic acid used as a blocking/quenching agent in the binding reaction to minimize the binding of nonspecific proteins to the labeled target DNA. The most common nonspecific competitors used in DNA gel shift assays are sonicated salmon sperm DNA and poly(dI•dC). These repetitive fragments, or polymers, provide an excess of nonspecific sites to adsorb proteins in crude lysates that will bind to any general DNA sequence.
- The specific competitor typically has the identical sequence as the labeled probe or contains a known consensus binding sequence for the target protein. Generally, a 200-fold molar excess of unlabeled probe is sufficient to out-compete any specific interactions with the same labeled probe, eliminating or reducing any positive shift results.

Traditional, radioactive EMSA protocols can be easily adapted to near-infrared fluorescence EMSA detection by using IRDye® end-labeled oligonucleotides and imaging with the Odyssey CLx or Odyssey Classic Infrared Imaging System, providing a safe and sensitive alternative.

EMSAs using near-infrared fluorescence technology are used to study:

- Regulation of transcription
- DNA replication

- DNA repair
- RNA processing



5.Pull down assay:

Pull-down assays are used to selectively extract a protein–DNA complex from a sample.

Pull-down assay uses a DNA probe labeled with a high affinity tag, such as biotin, which allows the probe to be recovered or immobilized.

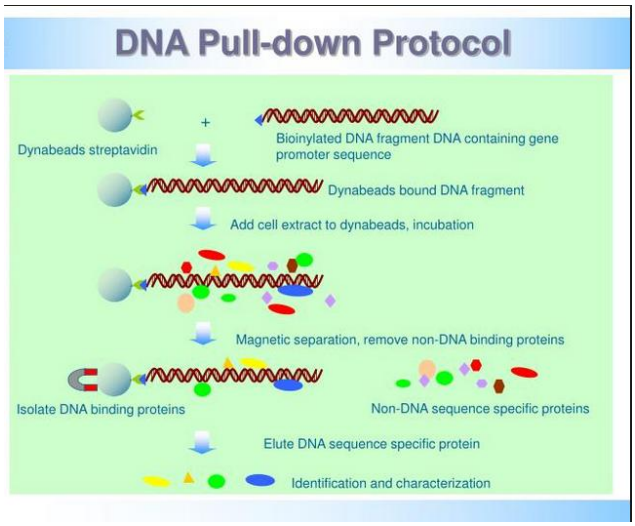
A biotinylated DNA probe can be complexed with a protein from a cell lysate in a reaction similar to that used in the EMSA and then used to purify the complex using agarose or magnetic beads.

The proteins are then eluted from the DNA and detected by western blot or identified by mass spectrometry.

Alternatively, the protein may be labeled with an affinity tag, or the DNA–protein complex may be isolated using an antibody against the protein of interest (similar to a supershift assay). In this case, the unknown DNA sequence bound by the protein is detected by Southern blotting or through PCR analysis.

Pull down assay advantages and disadvantages:

Strengths	Limitations
• enrichment of low abundant targets • end-labeled DNA can be generated by several methods • isolation of intact complex • compatible with immunoblotting and mass spectrometry analysis	• long DNA probes can show significant nonspecific binding • requires very specific antibodies for native proteins • requires nuclease-free conditions • assay must be performed in vitro



The protein isolated can be identifies The size, purity/concentration, and overall probe design is paramount to the success of the assay.

Factors effecting pull down assay:

DNA SIZE: Usually one Streptavidin molecule binds to four biotin molecules, steric hindrance can be problematic. Small probes (<100bp) can cause the potential binding site(s) to be too close to Streptavidin beads and thus may also restrict protein binding. Hence a probe size of 125–425bp is optimal for assay success.

DNA PURITY AND CONCENTRATION : DNA bait should be free of nucleases, to prevent degradation. Moreover, solutes should not be carried over from probe production procedures, as they may interfere with binding.

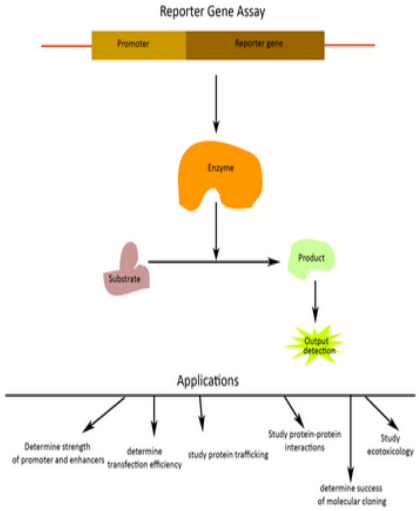
To ensure bead saturation, we recommend a probe concentration of 200–450ng/ul.

PROBE DESIGN : When constructing DNA bait for the affinity chromatography assay, one should consider the type of probe, the location of the biotin moiety, and the location of the potential binding site(s) of interest.

For ds DNA, since the biotin will interact with the Streptavidin bead, the biotin needs to be located on the 5’ end of an oligonucleotide.

A ssDNA probe is not constrained to one particular end and may be modified at either the 3’ or 5’ end.using western blot or mass spectrometry

6. Reporter assay:



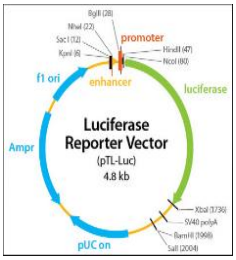
- Reporter gene assays are paramount for study of regulation of gene expression by gene regulatory elements:
- (cis-acting factors), transcription factors

(trans-acting factors) exogenous regulators

- The activity of a reporter gene is measured.
- A reporter gene is joined to a target regulatory DNA sequence

in an expression vector, which is then transfected into the cell type

of choice.



- The reporter gene is transcribed and translated

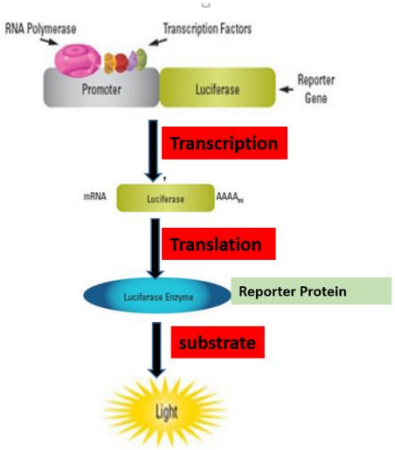
in the cells and its activity is measured

- The activity of the reporter gene can help us to understand

❑ strength or function of the target regulatory DNA sequence

❑ Binding of a transcription factors

effect of potential drugs and other ligands on transcription factor binding



Light/luminescence signal= Luciferase
Expression=Promoter activity

Examples of Reporter genes:

Commonly used reporter gene that fits the definition, widely available and commonly used are:

- β-galactosidase [*β-Galactosidase Assay (CPRG), Fluorescent β-Galactosidase Assay (MUG)*]
- β-glucuronidase (GUS assay used mostly for expression in plants)
- Luciferase (*Lumino™ Firefly Luciferase Assay*)
- Green fluorescent protein (GFP)
- Secreted Placental Alkaline Phosphatase

Characteristics of Reporter genes:

The important features of a good reporter gene are:

(1) It encodes a protein which is not normally present in mammalian cells

(2) Encoded protein is biologically active when translated so that its activity can be measured.

Furthermore, the choice of reporter assay also depends on:

- ✓ Type of study (gene regulation or measuring transfection efficiency)
- ✓ Organism and cell type
- ✓ Type of result sought (spatial or temporal)
- ✓ Detection method (histochemical staining, scintillation counters, spectrometry, fluorimetry or luminometry).

Widespread applications of reporter gene assays

Reporter gene constructs and the reporter gene assays find widespread applications in scientific research:

- ***Characterize the strength of promoters and enhancers***.

To study the strength of promoter or enhancer, the reporter gene is cloned downstream of promoter in a vector and then after transfection, its activity is measured by monitoring expression of the reporter protein. If the expression of reporter gene is high then the promoter is strong and if expression is low then promoter is weak. Similarly, the strength of enhancer region which is upstream of promoter is determined. If the transcription factors when bound to enhancer activate or increase the expression of reporter protein, then the enhancer is strong.

- ***Help in defining or characterizing role of various transcription factors***.

Transcription factors play a key role in signal transduction as they are trans-acting factors which bind to the enhancer region of the DNA upon receiving signal from cell and results in increase or decrease in expression of genes. The effect of transcription factor is multifaceted and its difficult to study its role in cells without using any molecular tool. Reporter genes assay help to alleviate this as one can study effect of transcription factor on desired regulatory sequence or gene expression by cloning the reporter gene downstream of regulatory DNA sequence in a vector and then transfecting vector in cell type or cell line where the target transcription factor is expressed.

- ***Measures transfection efficiency and the success of molecular cloning.***

To measure the transfection efficiency, reporter gene is cloned downstream of a constitutive promoter and then co-transfected along with the experimental plasmid. Expression of reporter protein shows the success of transfection and its quantification is a measure of transfection efficiency. Similarly success of molecular cloning can be monitored by reporter protein expression which is fused with the desired protein.

- ***Monitoring expression of gene and protein trafficking***

Reporter gene fused with gene of interest can help in monitoring expression of the gene and trafficking of the protein as reporter gene can be detected easily.

- ***Reporter gene assay in ecotoxicology***

Reporter gene assays also find application in ecotoxicology for accessing the potential toxic effects of environmental samples. These reporter gene assays are based on tester cells or bioreporters which are genetically engineered to report small toxicity by producing a high quantifiable signal.

7. CHROMATIN IMMUNOPRECIPITATION (ChIP)

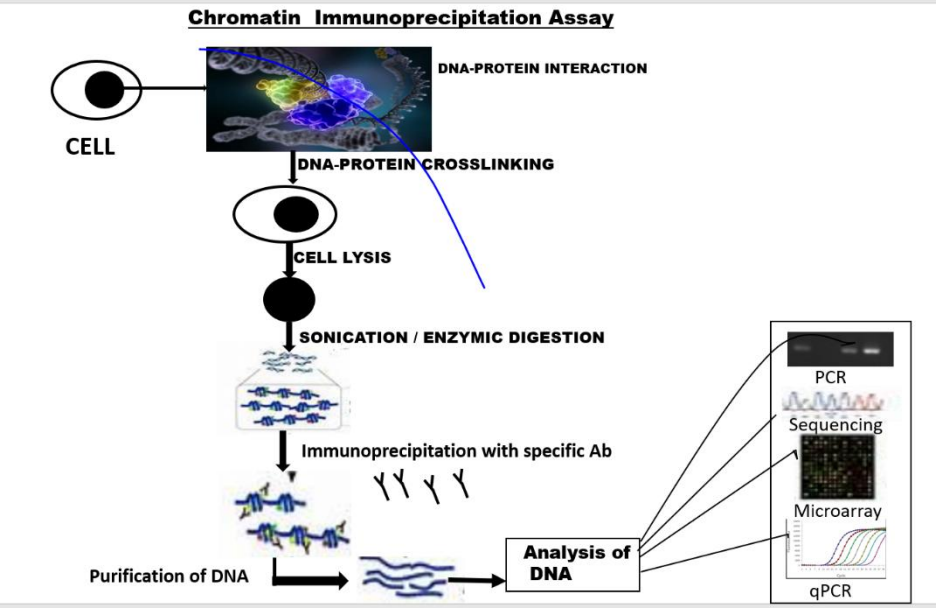
- Chromatin immunoprecipitation, or ChIP, refers to a procedure used to determine whether a given protein binds to or is localized to a specific DNA sequence *in vivo*.
- ChIP can also combine with paired-end tags sequencing in Chromatin Interaction Analysis using Paired End Tag sequencing (ChIA-PET), a technique developed for large-scale, de novo analysis of higher-order chromatin structures

OVERVIEW OF ChIP PROCESS:

- DNA-binding proteins are crosslinked to DNA with formaldehyde in vivo.
- Chromatin is isolated and DNA is sheared along with bound proteins into small fragments.
- Bind antibodies specific to the DNA-binding protein to isolate the complex by precipitation. Cross-linking is reversed to release the DNA and the protein is digested using Proteinase K
- The purified DNA sequences bound by the protein of interest can be further analyzed using ChIP-PCR, ChIP-microarray or ChIP-Sequencing

Native ChIP:

- ✓ Native ChIP is mainly suited for mapping the DNA target of histone modifiers.
- ✓ Native chromatin is used as starting chromatin.
- ✓ As histones wrap around DNA to form nucleosomes, they are naturally linked.
- ✓ Chromatin is sheared by micrococcal nuclease digestion, which cuts DNA at the length of the linker, leaving nucleosomes intact and providing DNA fragments of one nucleosome (200bp) to five nucleosomes (1000bp) in length.



- ✓ The purified DNA can be analyzed using PCR, microarray and sequencing

Crosslinked ChIP(X-ChIP)

- ✓ Cross-linked ChIP is mainly suited for mapping the DNA target of transcription factors or other chromatin-associated proteins, and uses reversibly cross-linked chromatin as starting material.
- ✓ Reversible cross-linking could be performed using formaldehyde or UV light.
- ✓ Then the cross-linked chromatin is usually sheared by sonication, providing fragments of 300 - 1000 base pairs (bp) in length.
- ✓ Mild formaldehyde crosslinking followed by nuclease digestion is also used to shear the chromatin at times

LIMITATIONS OF ChIP :

- The methodology of ChIP is highly dependent on the availability of ChIP grade antibodies or would need cells

and model organisms with epitope –tagged TF.

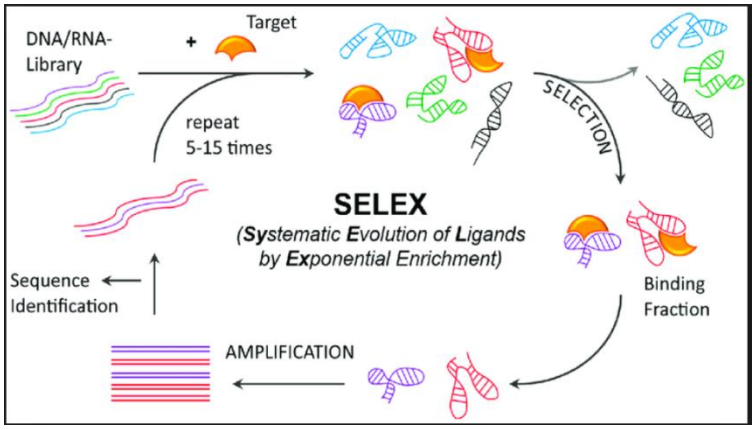
- Researchers studying differential gene expression patterns in small organisms also face problems as genes

expressed at low levels, in a small number of cells, in narrow time window.

- ChIP experiments cannot discriminate between different TF isoforms

8. Systematic evolution of ligands by exponential enrichment (SELEX)

- ➢ It is an *in-vitro* technique for producing oligonucleotides of either single-stranded DNA or RNA that specifically bind to a target ligand or ligands, which are commonly referred to as aptamers.
- ➢ The process begins with the synthesis of a very large oligonucleotide library consisting of randomly generated sequences of fixed length flanked by constant 5' and 3' ends that serve as primers
- ➢ For a randomly generated region of length *n*, the number of possible sequences in the library is 4^n (*n* positions with four possibilities (A,T,C,G) at each position)
- ➢ The sequences in the library are exposed to the target ligand - which may be a protein or a small organic compound. The sequences in the library are exposed to the target ligand - which may be a protein or a small organic compound.
- ➢ The bound sequences are separated using affinity chromatography or target capture on paramagnetic beads
- ➢ The specific sequences are eluted and amplified using PCR.



9. Yeast One Hybrid Assay:

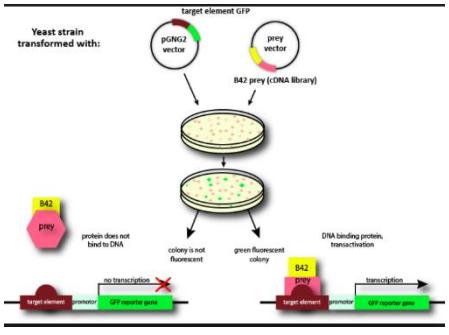
- Yeast one-hybrid (Y1H) assay is primary used to study what proteins bind a given DNA sequence *in vivo*.
- Y1H relies on the general principles of the yeast two-hybrid assay(Y2H), except that Y2H is used to study protein-protein interaction and Y1H is used to study DNA-protein interaction

WHY USE Y1H FOR DNA-PROTEIN INTERACTIONS:

1. Unlike luciferase reporter assays, Y1H is able to detect protein-DNA interactions that are not actually activating transcription. Fusing your protein(s) of interest to a strong activation domain allows Y1H to detect a variety of replication proteins, DNA repair proteins, and repressor proteins.

2. Y1H is compatible with many existing libraries. Most Y1H experiments can use hybrid prey libraries that have been constructed for Y2H applications, e.g. Gal4p- or LexA-based protein libraries can also be used for screening against various DNA baits in Y1H.

3. Y1H can detect isoform-specific interactions. You can design your prey plasmids to encode specific protein isoforms, allowing detection of highly specific interactions. In contrast, ChIP-based approaches rely on sensitive and specific antibody binding to your protein of interest, and these are rarely able to be achieved on an isoform-specific level.



Transcription factors or other DNA-binding proteins, expressed from cDNA expression libraries, can be identified due to the interactions with a DNA sequence-of-interest that is linked to a reporter gene, such as the yeast *HIS3* gene.

SUMMARY

1. Nucleic acid and protein interaction control key cellular processes, which includes both DNA and RNA protein interaction .

2. DNA-binding proteins(DBPs), such as transcription factors, constitute about 10% of the protein-coding genes in eukaryotic genomes and play very important role in the regulation of chromatin structure and gene expression by binding to short stretches of DNA.

3. RNA-binding proteins are proteins that bind to ribonucleic acid (RNA) molecules, which can be found

in the cytoplasm and nucleus, and are important in forming ribonucleoproteins (RNPs). A type of RNP, hnRNPs (heteronuclear proteins), are important in splicing of mRNA (messenger RNA), polyadenylation, stabilization, localization and translation.

4. During the late 19^{th} century, scientists microscopically observed the association of proteins with DNA strands.

The most successful method of visualizing nucleic acids (DNA and RNA molecules, DNA-RNA hybrids) was first reported by Kleinschmidt and Zahn (1959).

5. Electron microscopy is one of the first method to study nucleic acid –protein interaction. It captures interactions based on the existence of conformational structures such as kinks, bents, loops, etc., either on naked DNA, or on DNA associated with various proteins or ligands.

6. Other microscopic techniques include optical, fluorescence, and atomic force microscopy (AFM).

7. Structure determination using X-ray crystallography has been used to give a highly detailed atomic view of protein–DNA interactions.

8. *DNA footprinting* is a method of investigating the sequence specificity of *DNA*-binding proteins in vitro. The basis of this technique is that DNA bound proteins protect DNA from external agents that are known to cleave or modify DNA.

9. EMSA has been used widely in the study of sequence-specific DNA-binding proteins such as transcription 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.The assay is based on the observation that complexes of protein and DNA migrate through a non-denaturing polyacrylamide gel more slowly than free DNA fragments or double-stranded oligonucleotides.

10. Pull-down assays are used to selectively extract a protein–DNA complex from a sample.Pull-down assay uses a DNA probe labeled with a high affinity tag, such as biotin, which allows the probe to be recovered or immobilized.

11. Reporter gene assays are paramount for study of regulation of gene expression by gene regulatory elements.A reporter gene is joined to a target regulatory DNA sequence in an expression vector, which is then transfected into the cell type of choice. The reporter gene is transcribed and translated in the cells and its activity is measured .

12. Chromatin immunoprecipitation, or ChIP, refers to a procedure used to determine whether a given protein binds to or is localized to a specific DNA sequence *in vivo*.

The purified DNA sequences bound by the protein of interest can be further analyzed using ChIP-PCR, ChIP-microarray or ChIP-Sequencing .

13. Systematic evolution of ligands by exponential enrichment (SELEX) is an in-vitro technique. The process begins with the synthesis of a very large oligonucleotide library consisting of randomly generated sequences of fixed length flanked by constant 5' and 3' ends that serve as primers.

14. Yeast one-hybrid (Y1H) assay is primary used to study what proteins bind a given DNA sequence *in vivo*.

Transcription factors or other DNA-binding proteins, expressed from cDNA expression libraries, can be identified due to the interactions with a DNA sequence-of-interest that is linked to a reporter gene, such as the yeast *HIS3* gene.