DETERMINATION OF DNA SEQUENCE SPECIFICITY OF A DNA-BINDING PROTEIN.

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To show various biochemical techniques by which, the specific DNA sequence where a DNA binding protein binds to, can be ascertained.

To Understand Interference Assays

Discuss how Electrophoretic Gel Mobility Shift Assays, which in conjugation with Chemical synthesis of DNA, can be used to verify protein binding sequences of DNA.
DNA binding Proteins are important because

- Involvement in cellular processes such as
  - Replication
  - Recombination
  - Viral integration
  - Transcription
  - Others
DETECTION OF DNA SEQUENCES WHERE PROTEINS BINDS TO

- **DNA Footprinting Analysis/DNA Protection**
  - DNase Protection
  - DMS Protection.

- **PCR Based Techniques**

- **Interference Assays:**
  - Methyl Interference
  - Uracil Interference

- **Electrophoretic Gel Mobility Shift Assays** {EMSA}
Electrophoretic Mobility Shift Assay

- Method used to study protein DNA or protein RNA interactions.
- Are also called gel shift assay, gel mobility shift assay, band shift assay, and gel retardation assay.
- Electrophoretic separation of a DNA/protein mixture.
- Two lanes are used, one with the DNA/protein mix and another control of DNA alone.
- The gels are run for a period of time, and compared upon completion.
Electrophoretic Mobility Shift Assay

When the two lanes are compared there will be an extra band in the lane containing the less mobile DNA/protein mix.
An antibody can be added with affinity to a specific protein in a process called a super shift assay. It aids in identifying a specific protein if a combination of proteins are used.
DNAaseI FOOTPRINTING
DMS FOOTPRINTING ASSAY

Bind Protein

\[
\text{CH}_3 \quad \text{DMS} \quad \text{CH}_3 \quad \text{CH}_3
\]

Remove Proteins, Depurinate, break DNA at apurinic sites

Electrophoresis

Footprint
Determination of Protein-DNA Sequence specificity by PCR assisted binding site selection

1. **Oligonucleotide Pool + Extract**
   - Incubate
   - DNA-protein complexes

2. **Proceed to Reselect DNA (Total Four Selections)**
   - Immunoprecipitate
   - Wash
   - Recover DNA
   - PCR Amplify DNA

3. **Oligonucleotides Enriched for Protein-Binding Sequences**
   - Perform protein binding (mobility shift assay)
   - PCR Amplify Complexed DNA
   - Clone and Sequence Amplified DNA

4. **Clones of Individual Selected Binding-Site Sequences**
URACIL INTERFERENCE ASSAY

1. PCR with dNTPs and dUTP
2. Isolate protein-bound DNA (DNAs in which uracil residues decrease protein binding affinity are selected against)
3. Cleave at uracil residues using UNG and piperidine
4. Separate reaction products on a denaturing gel
Most protein preparations will contain both specific and non-specific DNA binding protein.

For a specific competitor, the same DNA fragment (unlabeled) as the probe can be used.

The non-specific competitor can be essentially any fragment with an unrelated sequence,
CHEMICAL SYNTHESIS OF DNA PROBES

- Glass support
- Monomer 1
- Monomer 2
- DMT
- Base 1
- Base 2
- Oxidation by I$_2$
- Removal of DMT by ZnBr$_2$
- Repeat process with monomer 3, monomer 4, etc.
- Oligonucleotide
Thank You......