Ch 3 - L 1.3

Transcription Factors

Characterizaton of Transcription Factors

- Analysis of DNA-binding activity
- Structural analysis – crystallography
- Analysis of trans-activation properties
- Identification of DNA response elements = TFBS
- Identification of genome-wide binding activity
- Identification of co-operating TFs
- Protein-protein interactions on DNA

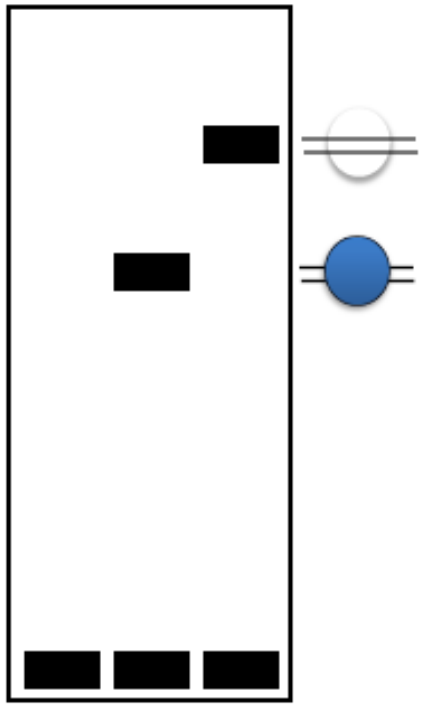
Characterizaton of Transcription Factors

- Analysis of DNA-binding activity

Gel Shifts/Electrophoretic Mobility Shift Assays

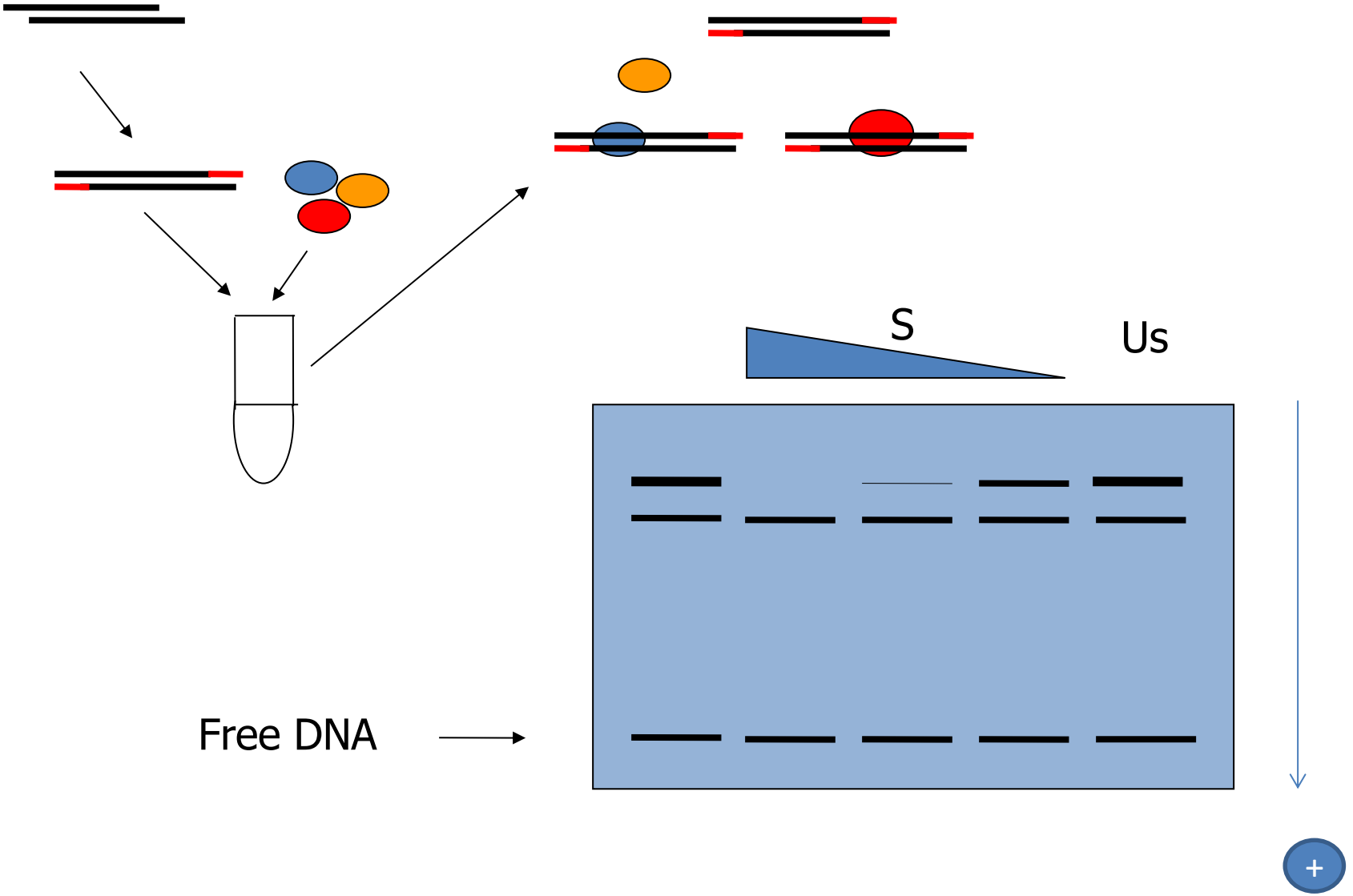
- In vitro analysis of the transcriptional factor binding function
- Binding does not always correlate with transcriptional activity

1. Nuclear extracts from cells or tissues
2. Mix with ^{32}P-labeled ds-oligo
3. Run on Native acrylamide gels



Free oligo

Gel-shift assay or Electrophoretic Mobility Shift Assay EMSA



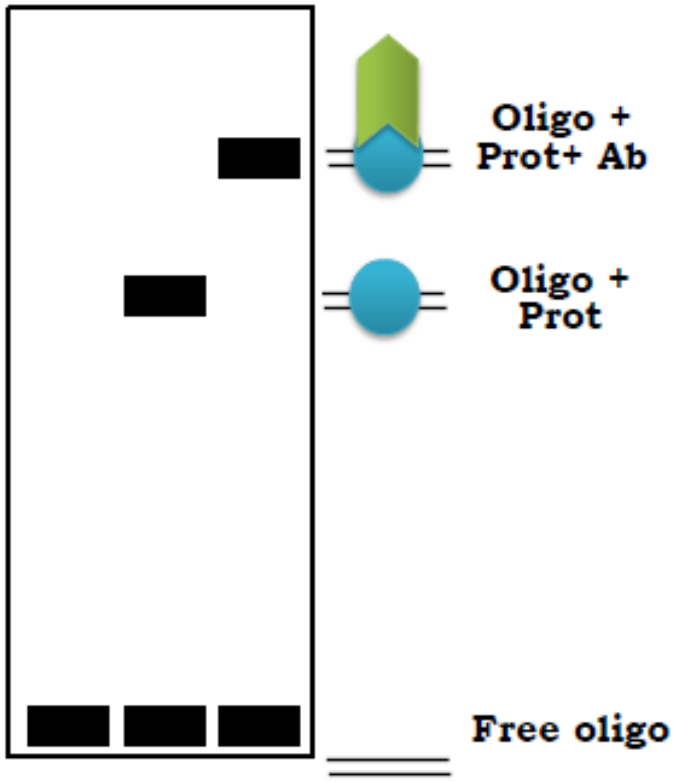
How do we determine the identity of complexes and if they are specific?

- **Competition assays**

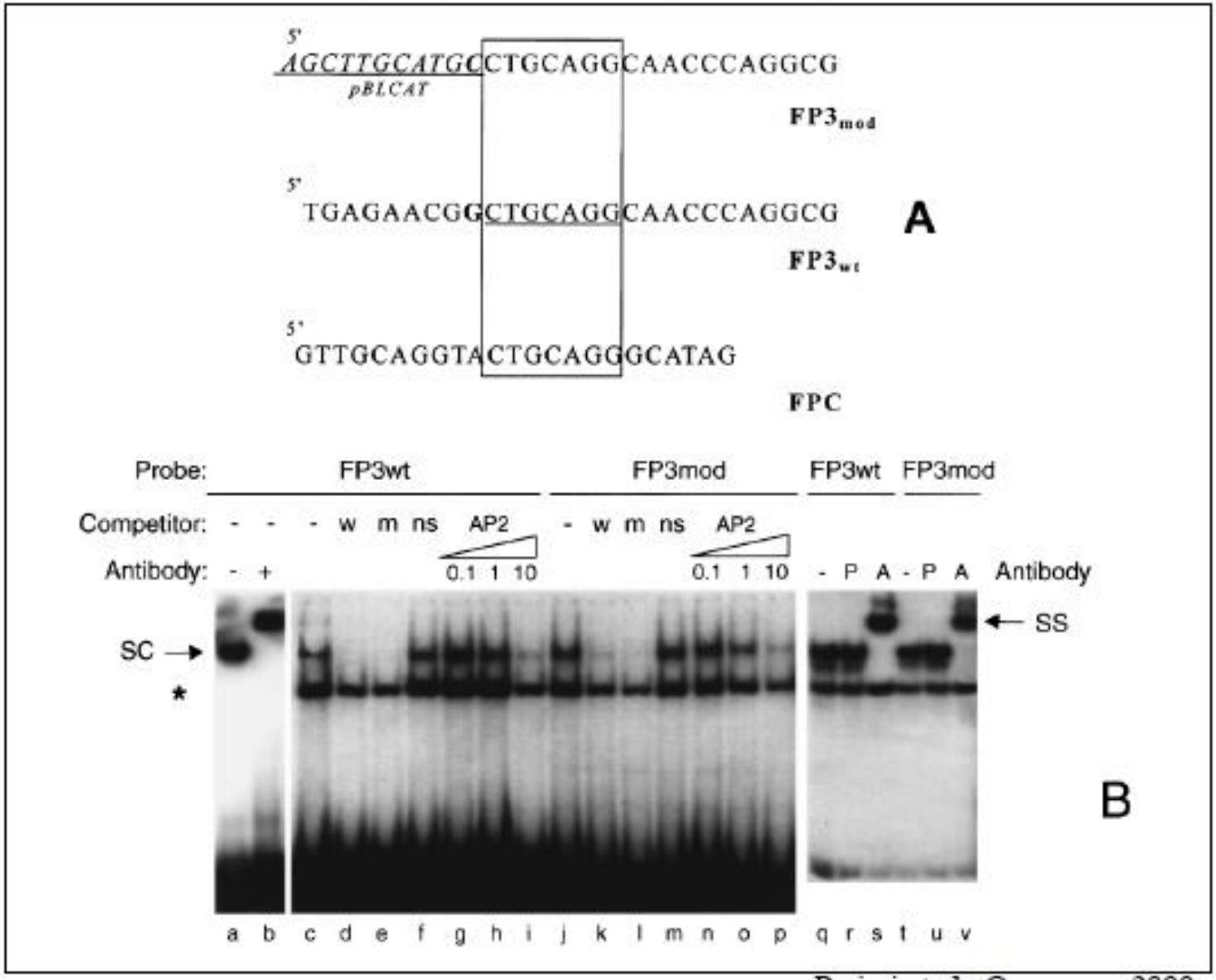
  Molar excess of identical, mutant, or consensus site

- **Supershift Assays**

  Add specific antibody to the binding assay



Examples: Gel Shifts/EMSA

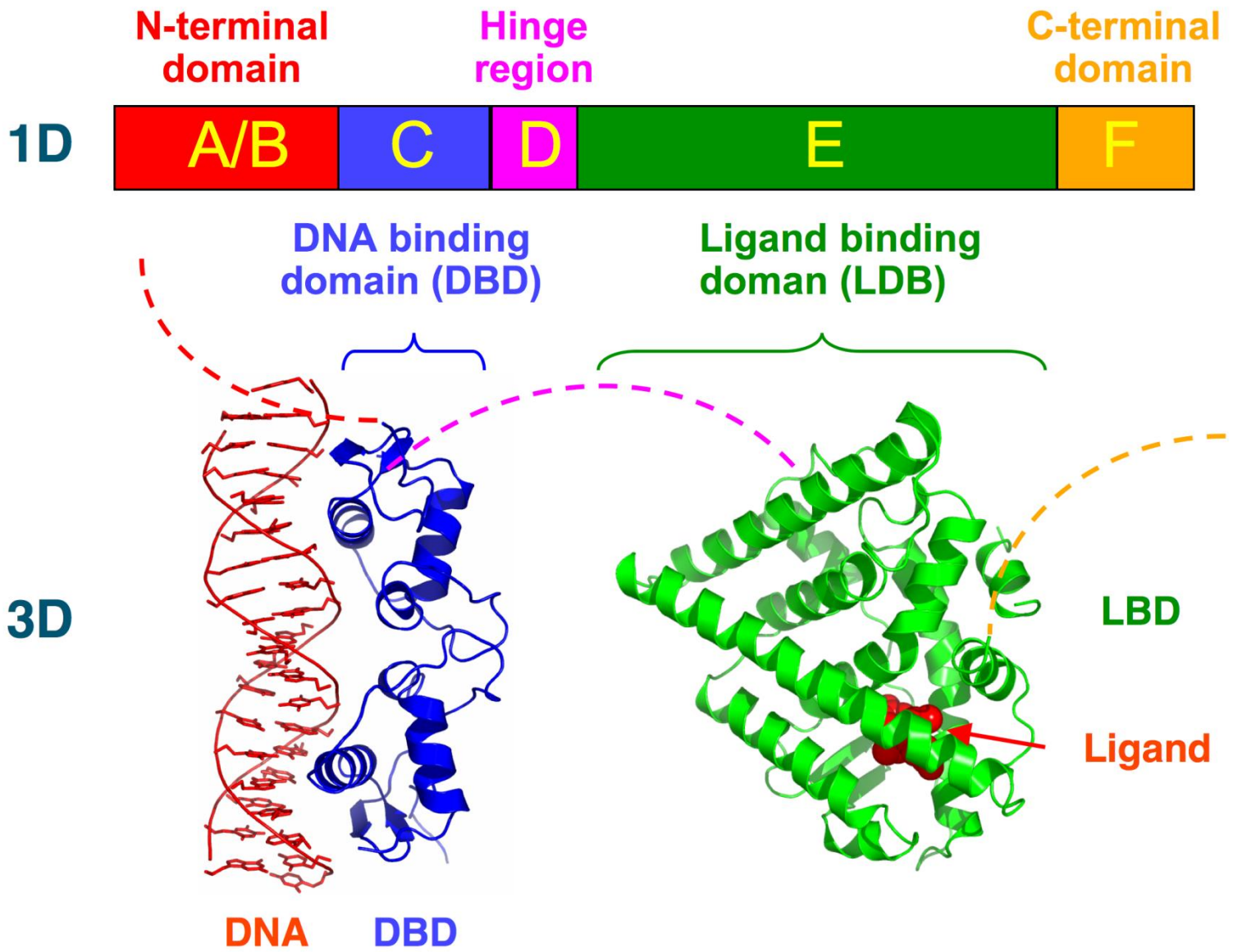


Perissi et al., Oncogene, 2000

Characterizaton of Transcription Factors

- Structural analysis – crystallography

Structural Organization of Nuclear Receptors

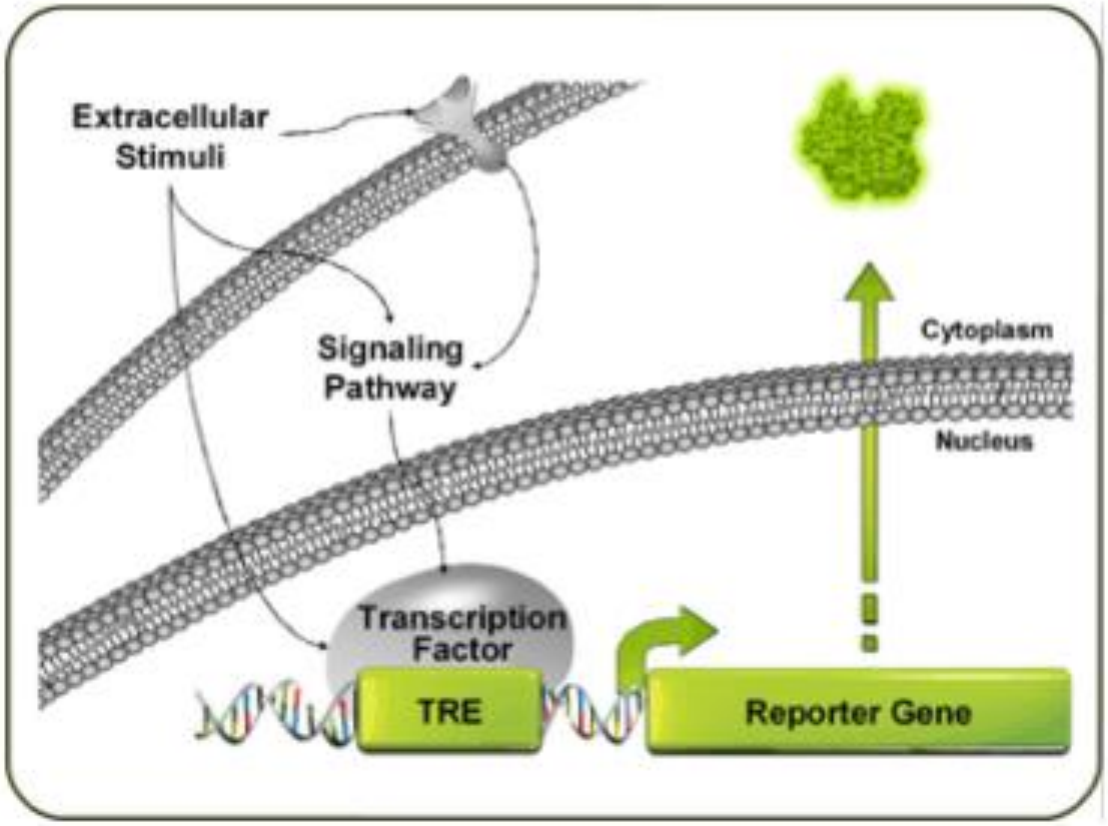


Structures (DBD)
http://www.rcsb.org/pdb/home/home.do

Characterizaton of Transcription Factors

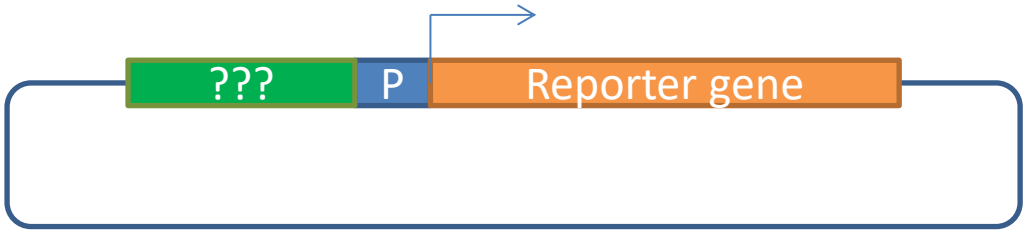
- Analysis of trans-activation properties

What is a Reporter Assay?



Functional validation: **reporter assay**

Plasmid with a **reporter** gene driven by a promoter: clone upstream studied fragment



Transfect into cultured cells, after 24-72 hours measure the product.

Reporter genes are usually nonmammalian genes:

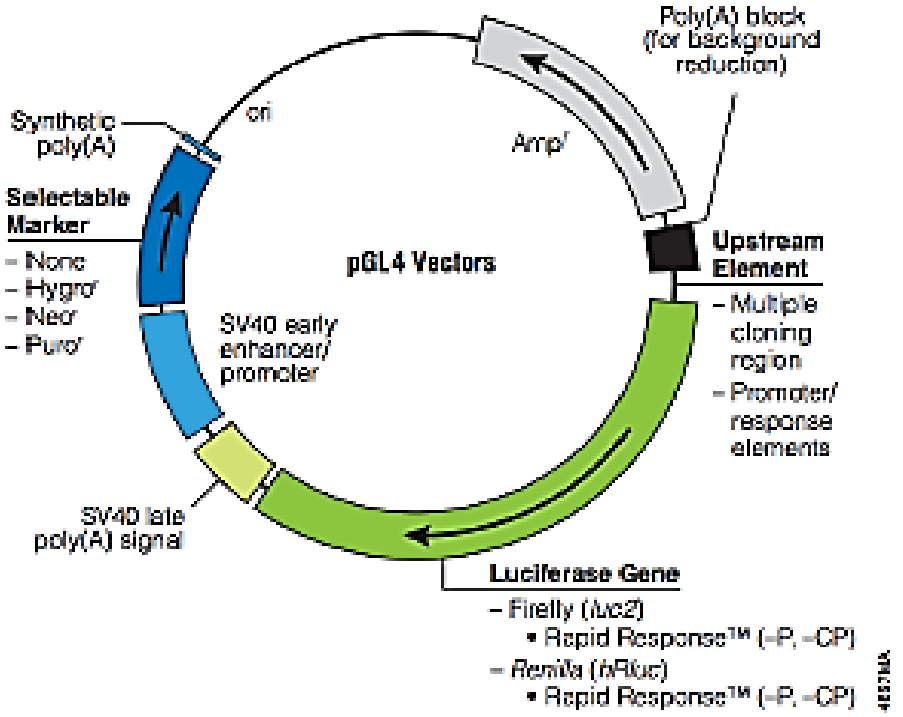
- CAT cloramphenicol acetyltransferase
- Luc firefly luciferase
- GFP Jellyfish green fluorescent protein
- β-gal beta-galactosidase

Deletional + Mutational analysis of fragment may lead to identify important elements

Luciferase Reporters—pGL Family

The pGL4 Vector family includes:

- Basic vectors with no promoter that contain a multiple cloning region for cloning a promoter of choice
- Vectors containing a minimal promoter
- Vectors containing response elements and a minimal promoter
- Promoter-containing vectors that can be used as expression controls or as co-reporter vectors



Reporter Assays

Strengths
High throughput
Can measure function of mutations in promoters
Large dynamic range

Many reporters possible
- GFP
- b-galactosidase
- CAT (chloramphenical acetyl transferase)
- Luciferase (firefly, renilla)

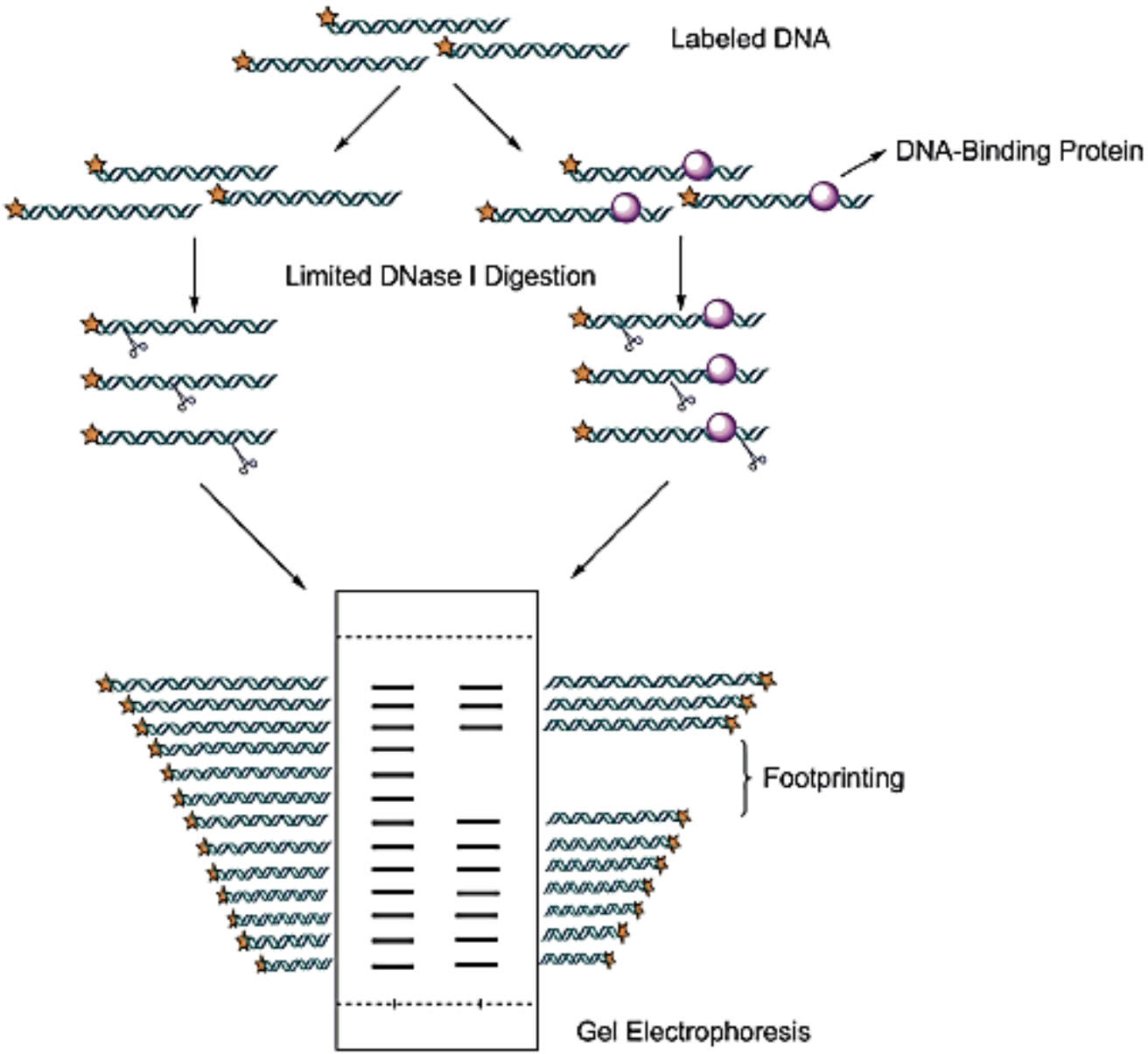
Can be used for in vivo/in cell monitoring.

Weaknesses
- uses exogenous DNA, not chromatin
- Gene dosage artifacts are possible
- relies on transfection, not easy for all cells

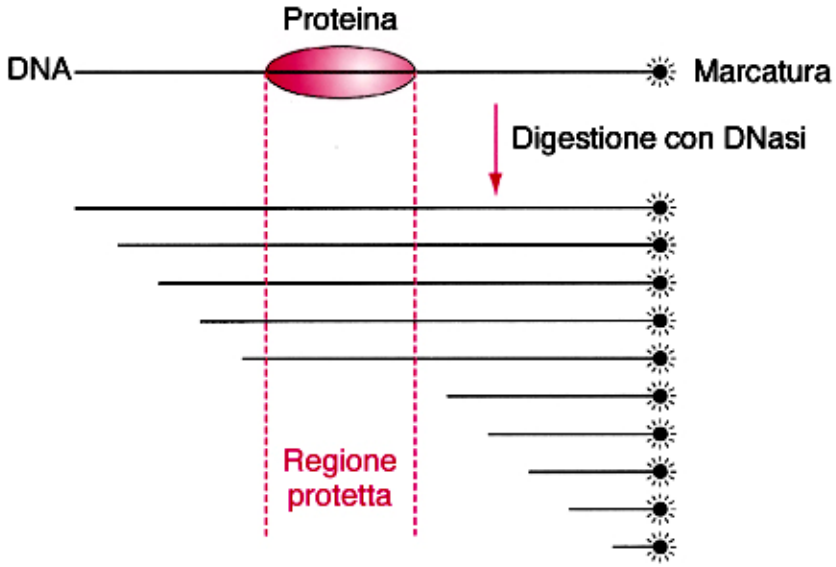
Characterizaton of Transcription Factors

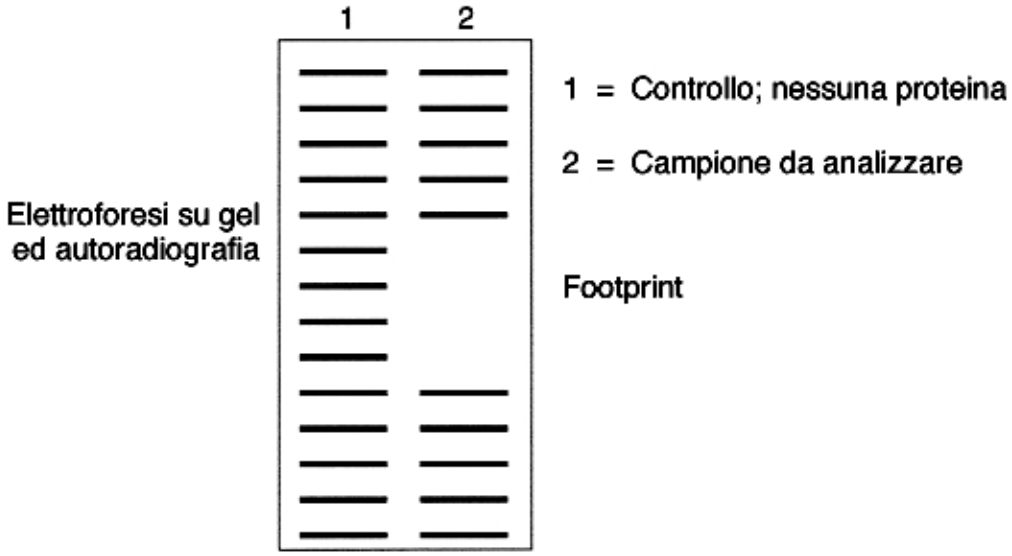
- Identification of DNA response elements = TFBS

DNaseI Footprinting



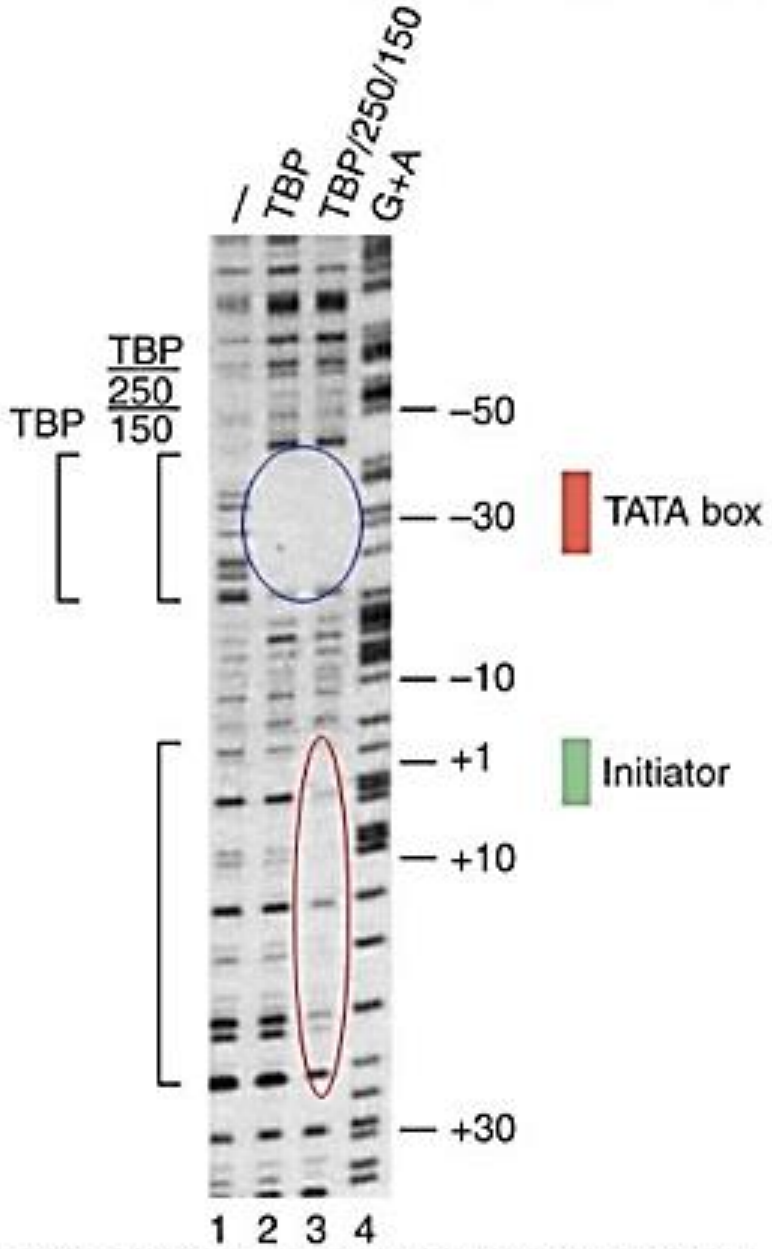
DNaseI Footprinting





DNaseI Footprinting of hsp70 promoter

$TAF_{II}250$ and $TAF_{II}150$ cooperate in binding to the initiator & DPE



Verrijzer et al., Promoter recognition by TAFs. *Cell* 81 (30 Jun 1995) p. 1117, f. 2c.
Reprinted by permission of Elsevier Science.

Identification of DNA Binding specificity

Historical

1° route:

isolating a promoter sequence, make deletional mutants and identify regulatory elements.

This is paralleled with Dnase I footprinting experiments using whole Nuclear Extract.

Once identified, the response elements are further analyzed by Band-shift (EMSA)

Proteins bound are then isolated by DNA affinity chromatography and identified.

This approach has led to the characterization of several tens of Transcription Factors.

2° route:

Several putative TFs are identified by homology cloning.

The binding site was often identified by **SELEX**

Finally, bioinformatic search for the binding site is performed on known genomic sequences.

3° route:

Conserved, nontranscribed sequences proximal to known genes are explored statistically to describe over-represented sequence “words” as compared to the whole genome.

Experimental proofs that the identified “words” (or motifs) can bind regulatory factors are needed

SELEX

A random sequence oligonucleotide library is explored using a purified or recombinant Transcription Factor

Classical SELEX: many rounds of selection + PCR amplification

SELEX variants: single selection step at high stringency, followed by elution and NGS sequencing

Usually produced a series of short sequences → consensus

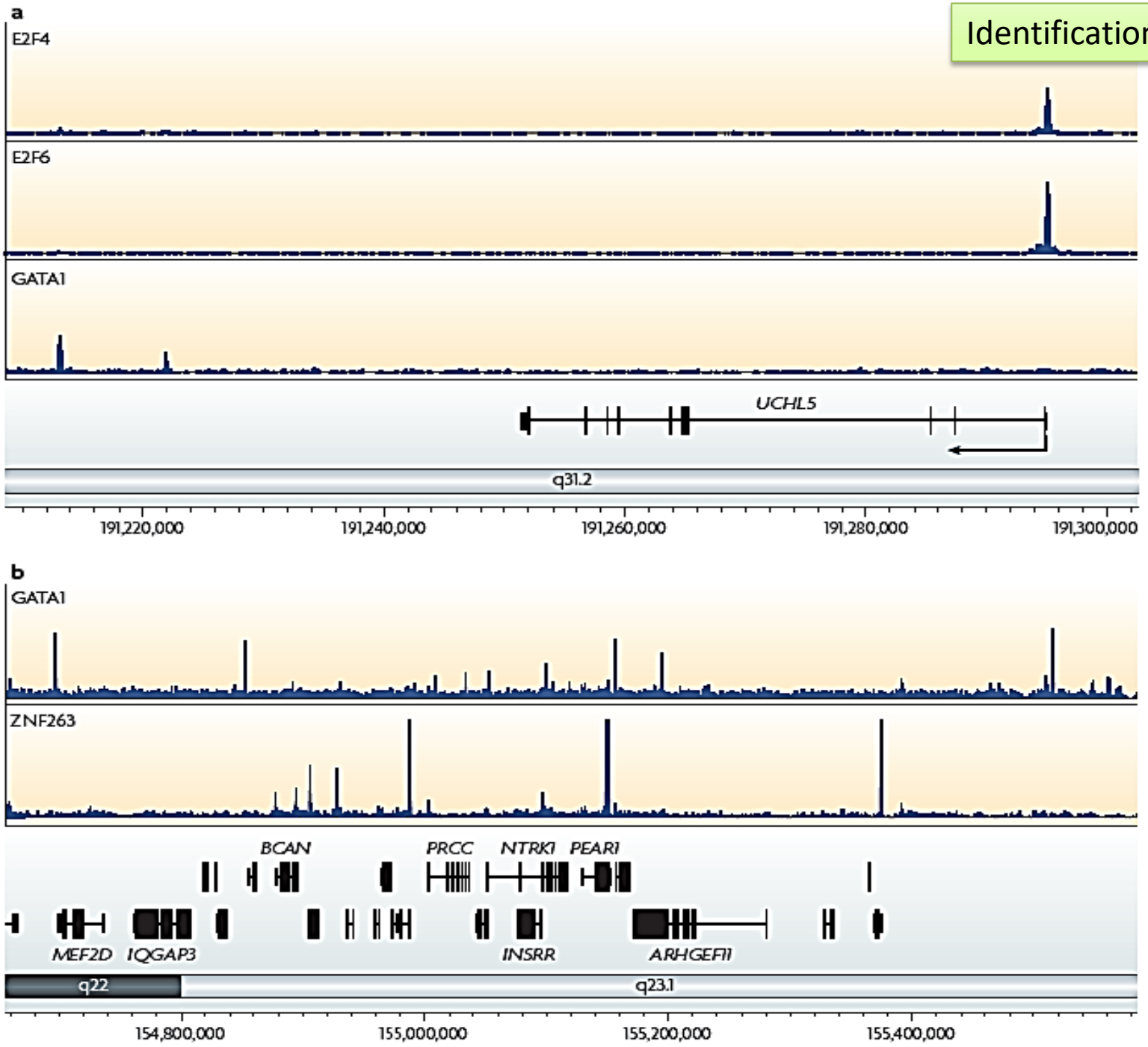
Using ChIP + microarrays or (best) ChIP-Seq it has been quite straightforward to obtain high-resolution maps of TF binding to chromatin using cell lines.

Insights from genomic profiling of transcription factors

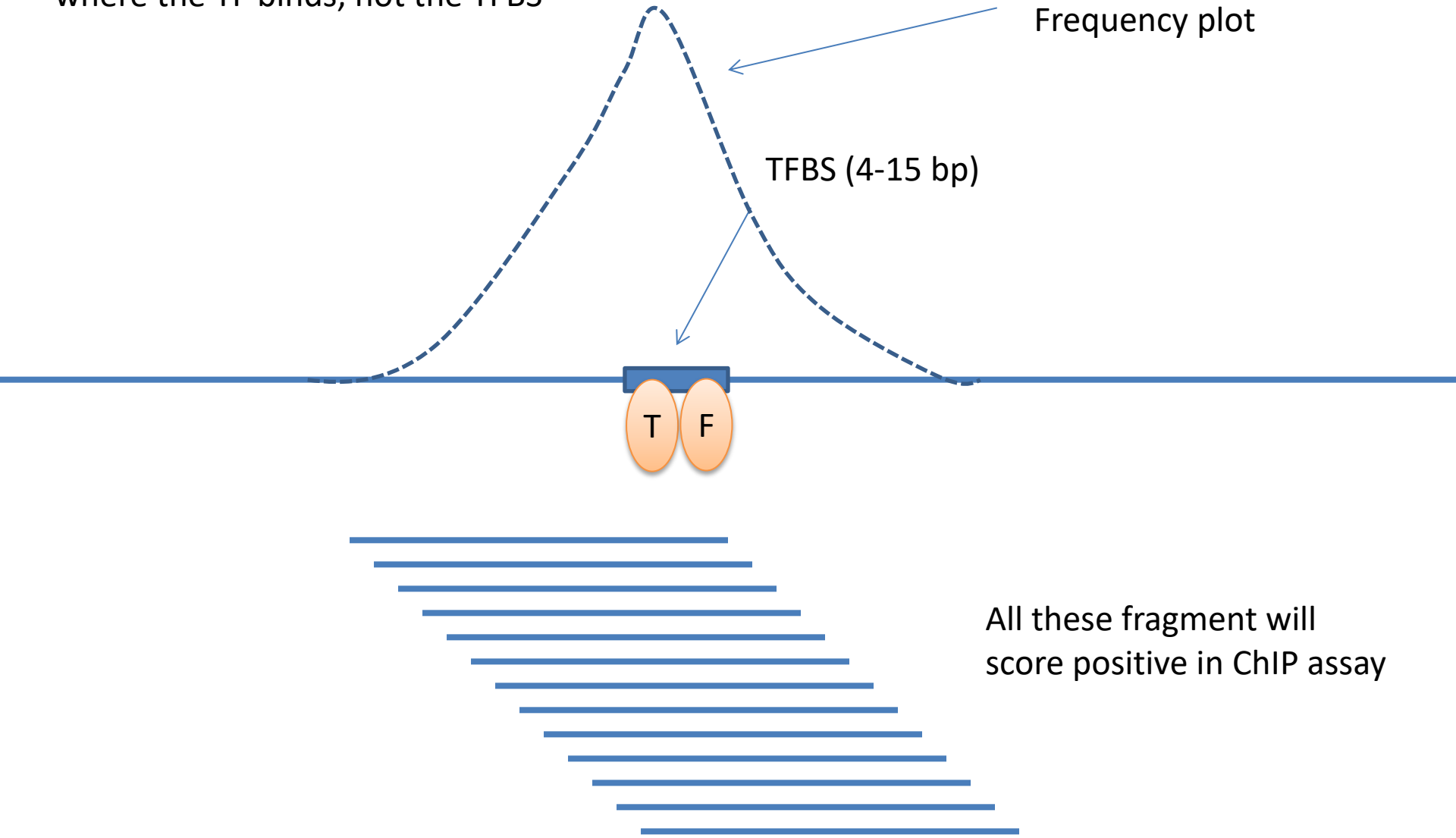
Peggy J. Farnham

Abstract | A crucial question in the field of gene regulation is whether the location at which a transcription factor binds influences its effectiveness or the mechanism by which it regulates transcription. Comprehensive transcription factor binding maps are needed to address these issues, and genome-wide mapping is now possible thanks to the technological advances of ChIP–chip and ChIP–seq. This Review discusses how recent genomic profiling of transcription factors gives insight into how binding specificity is achieved and what features of chromatin influence the ability of transcription factors to interact with the genome. It also suggests future experiments that may further our understanding of the causes and consequences of transcription factor–genome interactions.

Identification of R.E.



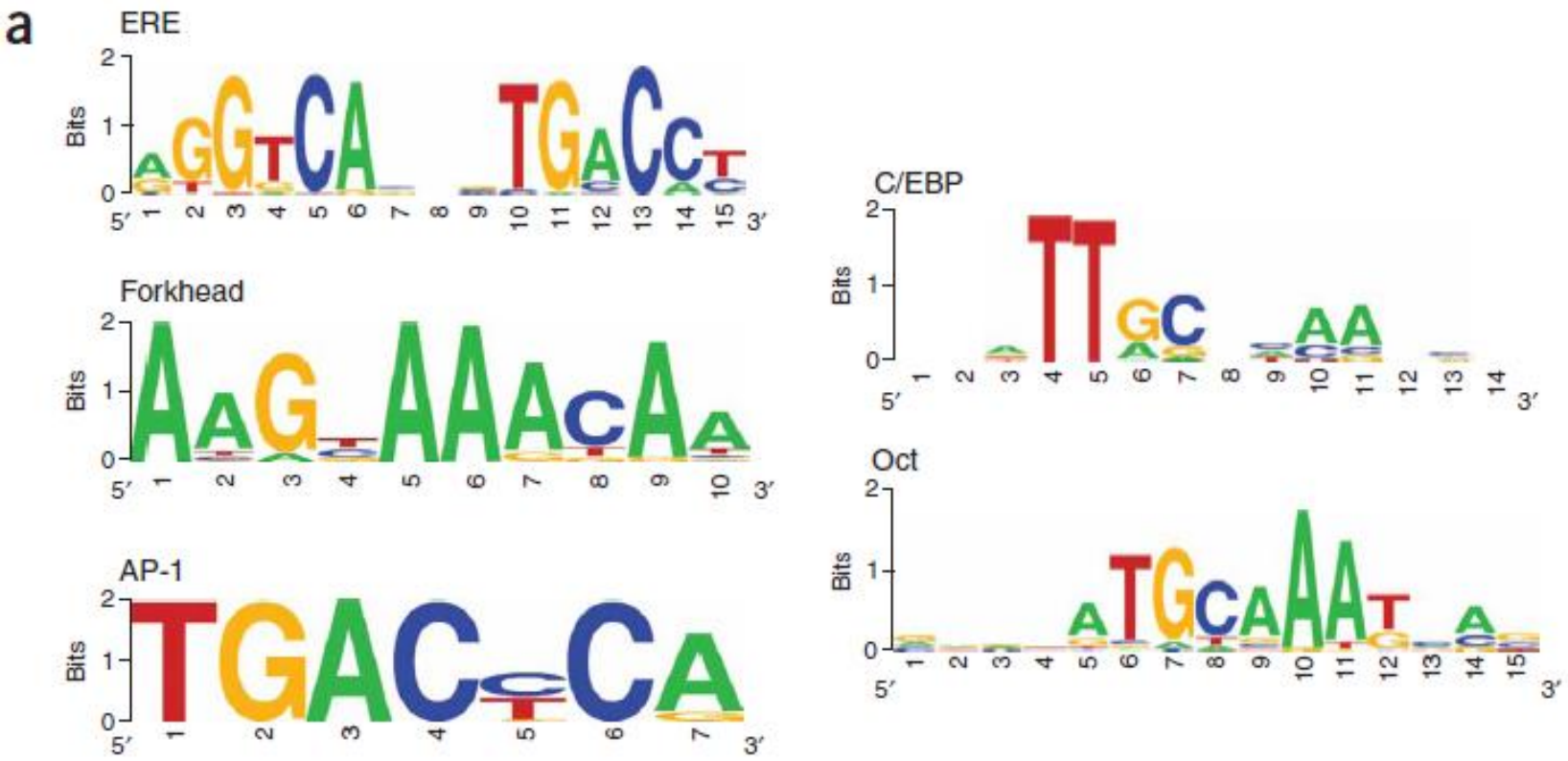
ChIp-Seq analysis identifies a region where the TF binds, not the TFBS



All these fragment will score positive in ChIP assay

In the **-500, +500** interval around binding peaks, algorithms exist to find unbiased overrepresented motifs, or known motifs based on **positional weight matrices**.

Examples:



Methodological Wiki

You can add subjects or tell me to add

Try to find images and explanations, with specific points, like these (as example):

Transcription Factors / DNA binding

Chromatin Immunoprecipitation

Overview:

Strengths

•Allows you to detect transcription factor binding at specific sites within chromatin in vivo in cells or tissues.

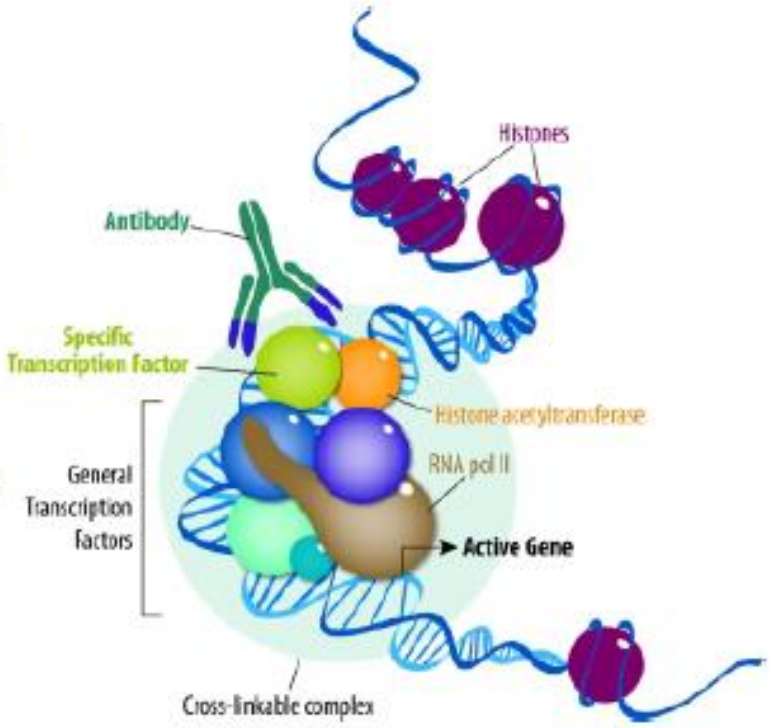
•Detection by PCR (qPCR) is very sensitive.

Weaknesses

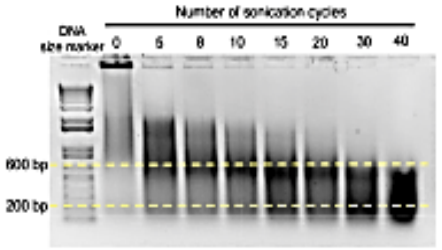
→ Requires long training and optimization steps

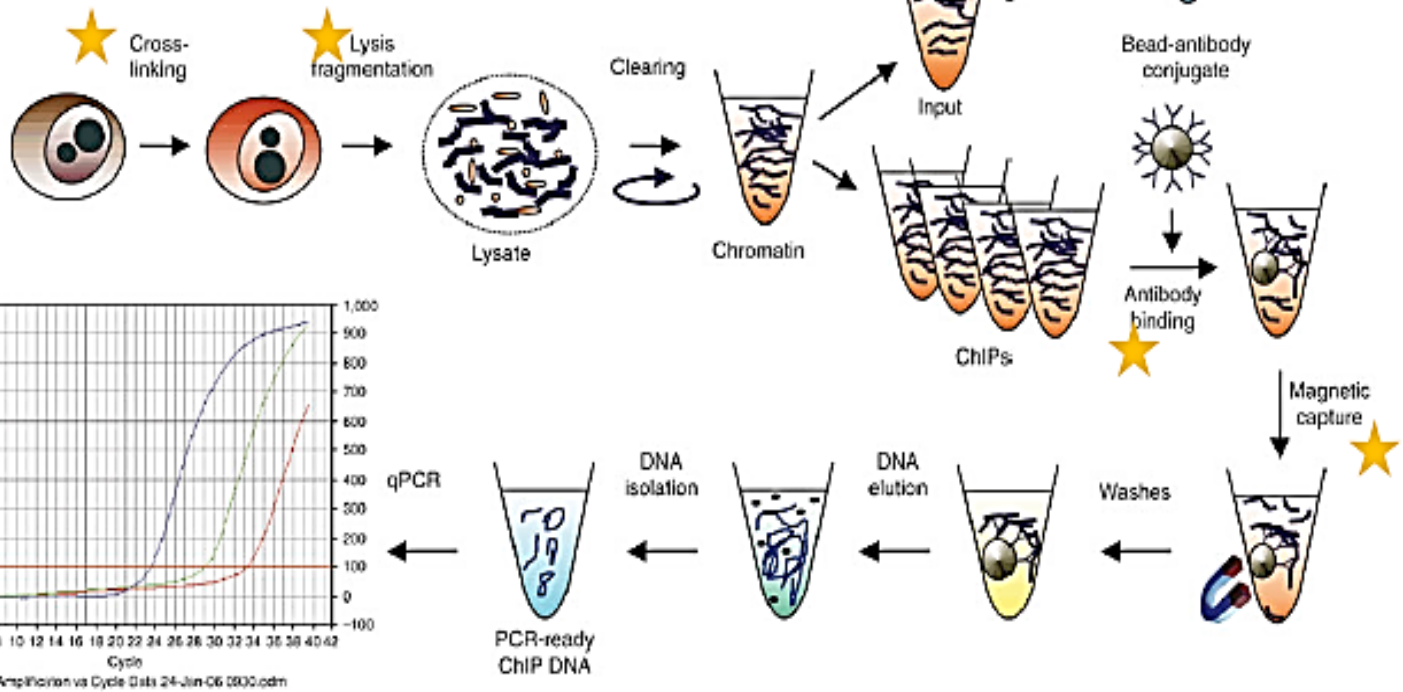
→ Requires very good antibodies (CHIP-grade)

→ does not exactly tell you where on DNA protein is binding.



ChIP Steps & Optimization





ChIP Controls

ChIP controls

- PolII or histone marks antibodies can be useful if you are unsure about your antibody (positive control for ChIP technique)
- Controls for genes that have previously been shown to be bound by factor if interest (positive control for antibody)
- Controls for unrelated genomic regions that should not bind factor of interest (negative control)
- Normal IgG or pre-immune IgG (negative control for IP)

PCR controls

- Negative PCR controls as usual
- Serial dilutions of input material to calculate reference curve
- Dissociation curve to validate primers