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Protein-DNA interaction

F1MG1_Metods of MB

Mgr. Denis Šubert
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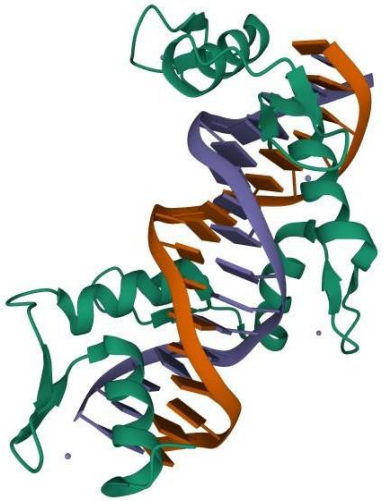
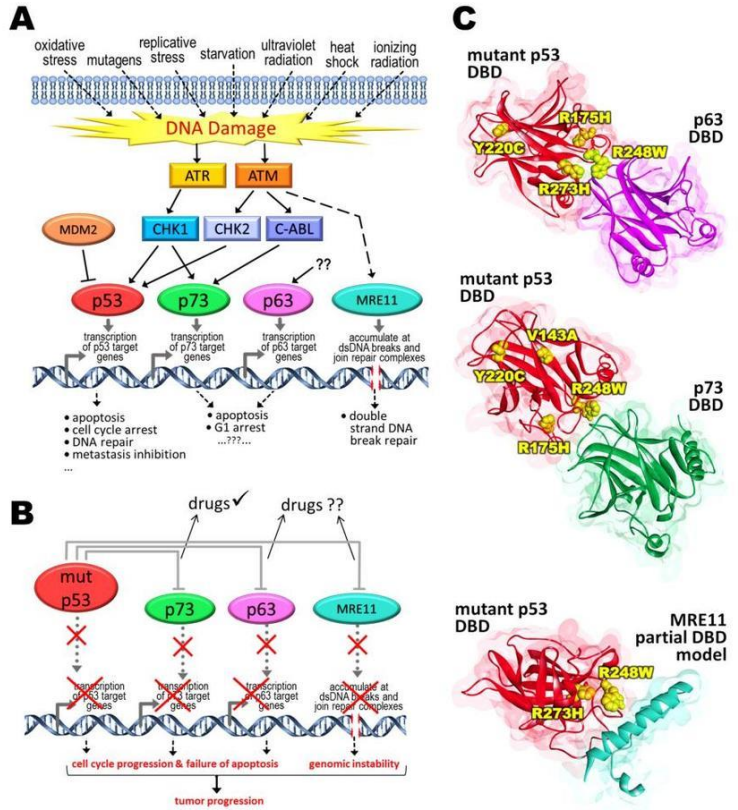
PDB id: 5KKQ, Hashimoto





https://www.researchgate.net/figure/Mutant-p53-proteins-carry-out-novel-oncogenic-interactions-A-Signaling-in-the-p53_fig2_277087873

Protein-DNA interactions

—Screening of interaction partners
—Localization of the interaction within the chromatin
—Sequential structural preference
—Protein function (TRF, Helicases, Chromatin, DNA-repair)

- A wide range of biophysical chemistry methods have been used to study interactions between proteins and nucleic acids.
- particularly good for determining the strength (affinity) of interactions
- High affinity, μM–nM: tend to involve sequential interactions,
- Low affinity, mM – μM: proteins tend to recognize aspects of the "whole" structure, ie.

DNA-protein binding motifs

Hydrogen bonds:

Adenine - Gln/ Asn

Guanine - Arg

Salt bridges:

Phosphate residue – Arg/ Lys

Through coordinately bonded metals

Zinc finger motif – Zn^{2+}

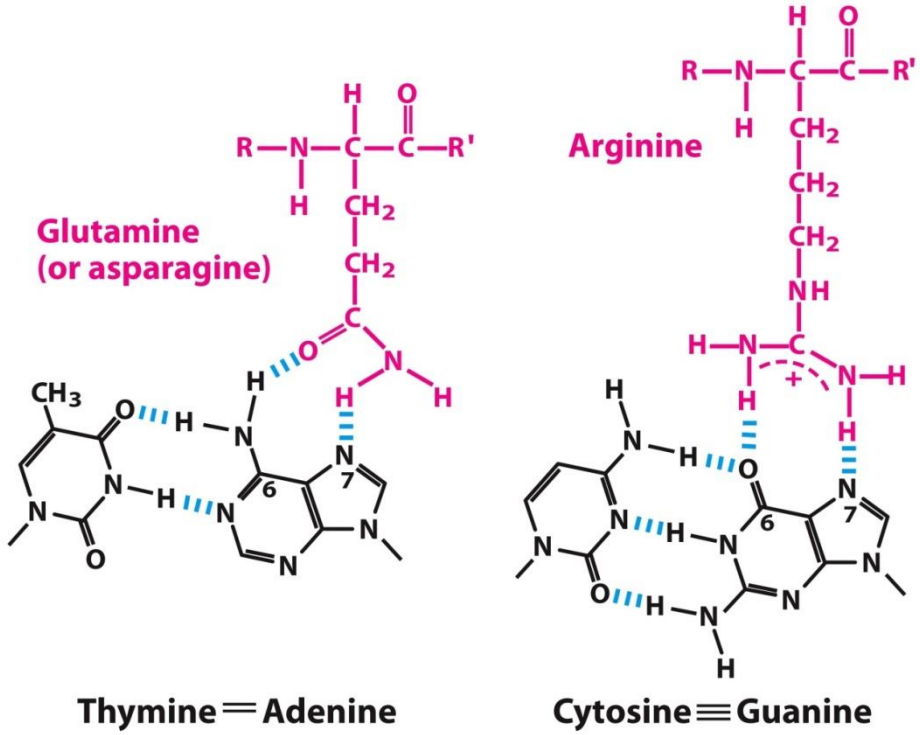


Figure 28-10
Lehninger Principles of Biochemistry, Sixth Edition
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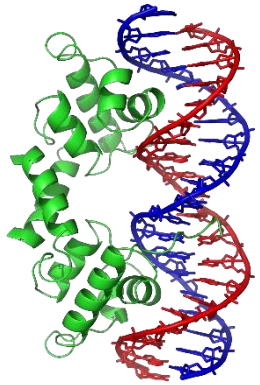
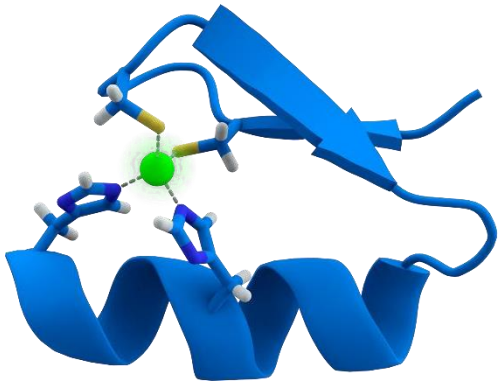
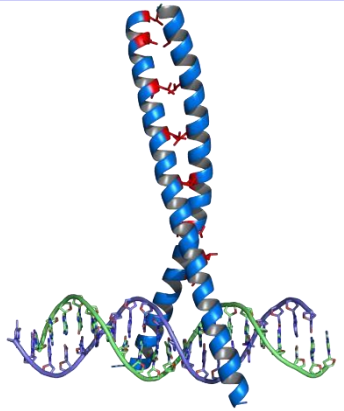
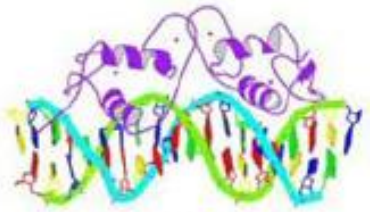
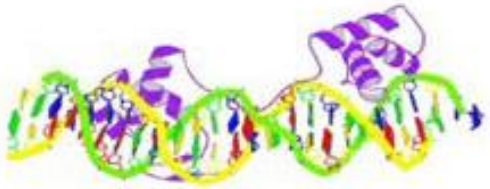
Gln/Asn forms specific H-bonds with N-6 H-7 H of Adenine

Arg forms specific bonds with the Cytosine-Guanine pair

The most common DNA-binding motifs

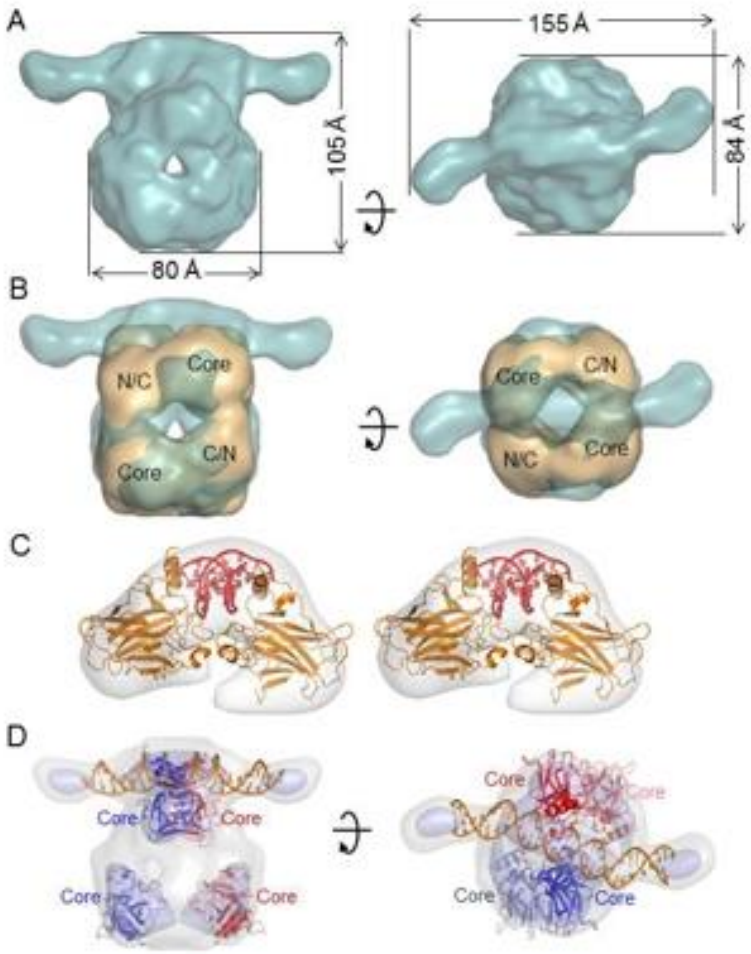
HTH	Zinc-Finger	Leucin zip
Binding to		
DNA major groove	DNA major groove	DNA major groove
Composition		
Helix-turn-Helix	ß-sheet-ß-sheet-Helix	Helix-Helix
Structure		
		

1. Basic domains  CREB (1DH3)	**2. Zinc-coordinating DNA-binding domains**  GR (1R4R)	**3. Helix-turn-helix domains**  Pax-6 (6PAX)
4. Other all-α-helical DNA-binding domains  SRY (1J46)	**5. α-Helices exposed by β-structures**  MEF2A (1C7U)	**6. Immunoglobulin fold**  NF-kappaB p50 (1SVC)
7. β-Hairpin exposed by an α/ β-scaffold  SMAD3 (1MHD)	**8. β-Sheet binding to DNA**  TBP (1CDW)	**9. β-Barrel DNA-binding domains**  YB-1 (1H95)

Methods for studying DNA-protein interactions

- Chip seq
Pull-down assay
EMSA
FRET
ITC
Co-crystallization
NMR
A yeast two-hybrid system
Gene expression

SELEX
database (interactome and complexes...)
genetic methods



DNA-protein interakce

In vitro methods	Description
Electrophoretic Mobility Shift Assay (EMSA)	The EMSA has been used extensively for studying protein:DNA interactions. The assay is based on the slower migration of protein:DNA complexes through a native polyacrylamide or agarose gel than unbound DNA. The individual protein:DNA complexes from discreet bands within the gel. Now, protein:RNA interactions can be detected with the first RNA-EMSA assay.
Supershift Assay	A variation of the EMSA that uses antibodies to identify proteins involved in the protein:DNA complex. The formation of an antibody:protein:DNA complex further reduces the mobility of the complex within the gel resulting in a "supershift."
Chromatin Immunoprecipitation (ChIP)	Captures protein DNA interactions via *in vivo* crosslinking. Antibodies are used to selectively precipitate a protein of interest, and the quantity of DNA bound to that protein is measured via Quantitative PCR.
Protein:DNA Crosslinking	Method for trapping protein:DNA interactions covalently under controlled conditions by labeling the protein bait and capturing the interacting DNA via coupling with a photo-reactive reagent. Excellent for capturing weak or transient interactions.
Affinity-based Methods	Uses labeled DNA or RNA fragments bound to an affinity support to capture or purify specific binding proteins from crude extracts.
DNA Footprinting	Method identifies the recognition site of a protein for a specific nucleic acid sequence. Binding of a protein to a specific DNA sequence protects that region of DNA from subsequent attack by DNase.
Reporter Assays	Identify gene promoter activity with reporter genes that are easily visualized. Provides real-time data in cell systems.

In Vivo Methods for Protein Interaction Analysis

In vivo methods for protein interaction analysis.

In Vivo Methods	Description
Yeast Two-Hybrid System	Monitor complex formation through transcriptional activation of reporter genes.
Crosslinking Reagents	Incorporating functional groups into proteins which can react, trapping a protein complex.
Immunofluorescence/FRET	Detect co-localized signal from two different proteins or monitor complex formation thought fluorescent resonance energy transfer.

Detection of DNA/RNA binding sites

ChIP (chromatinová imunoprecipitace)

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 specific transcription factors or histone proteins and to look at the precise genomic locations of various histone modifications (including acetylation, phosphorylation, or methylation).

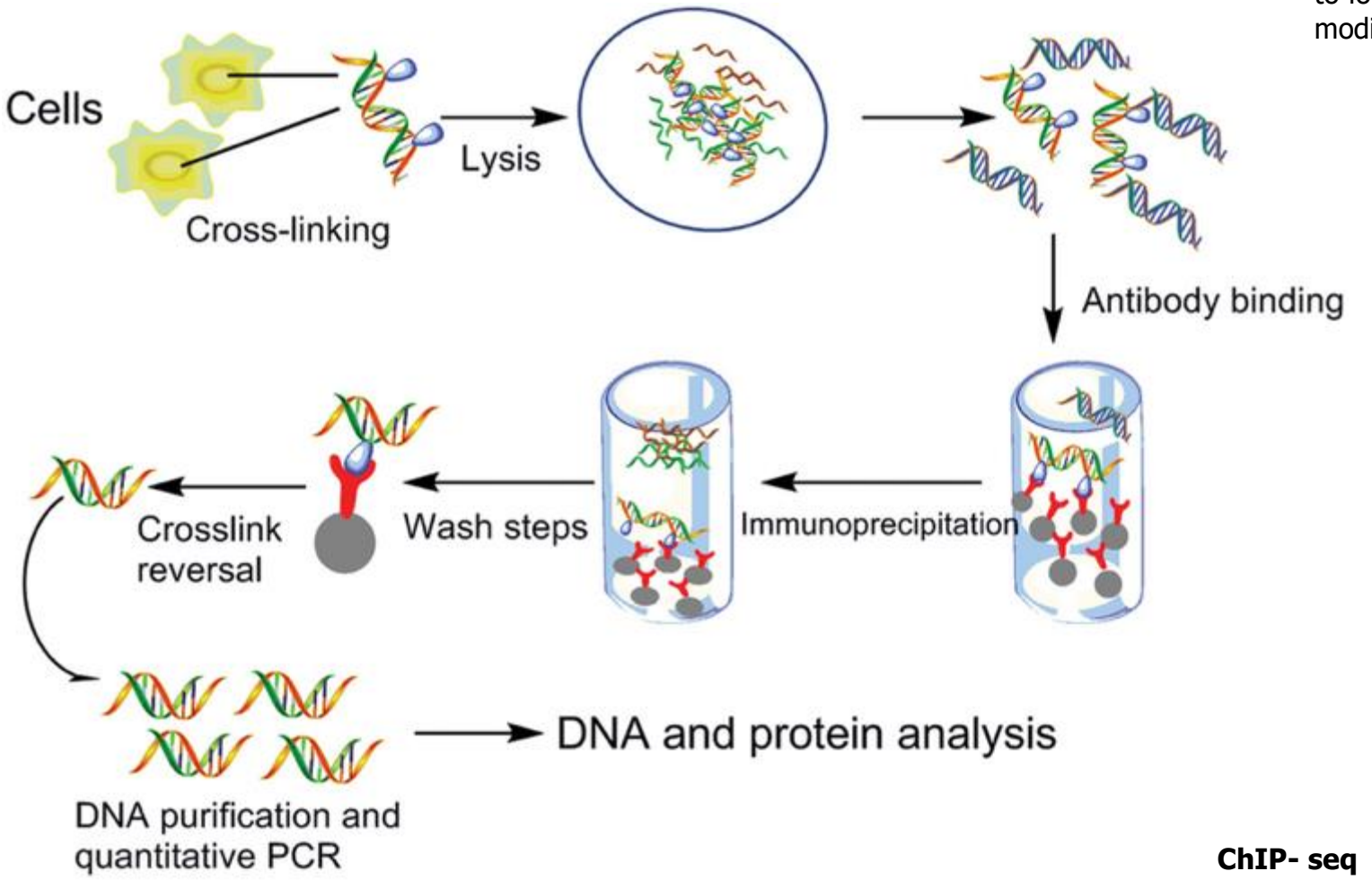
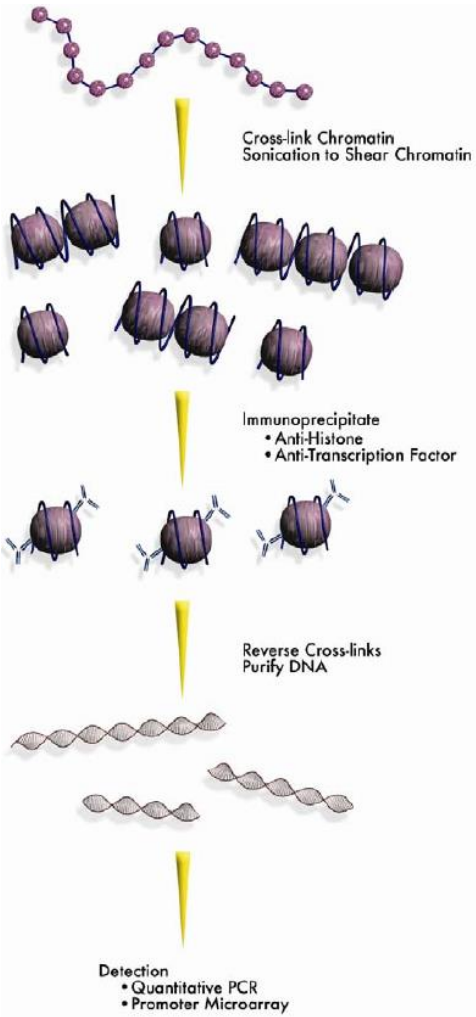
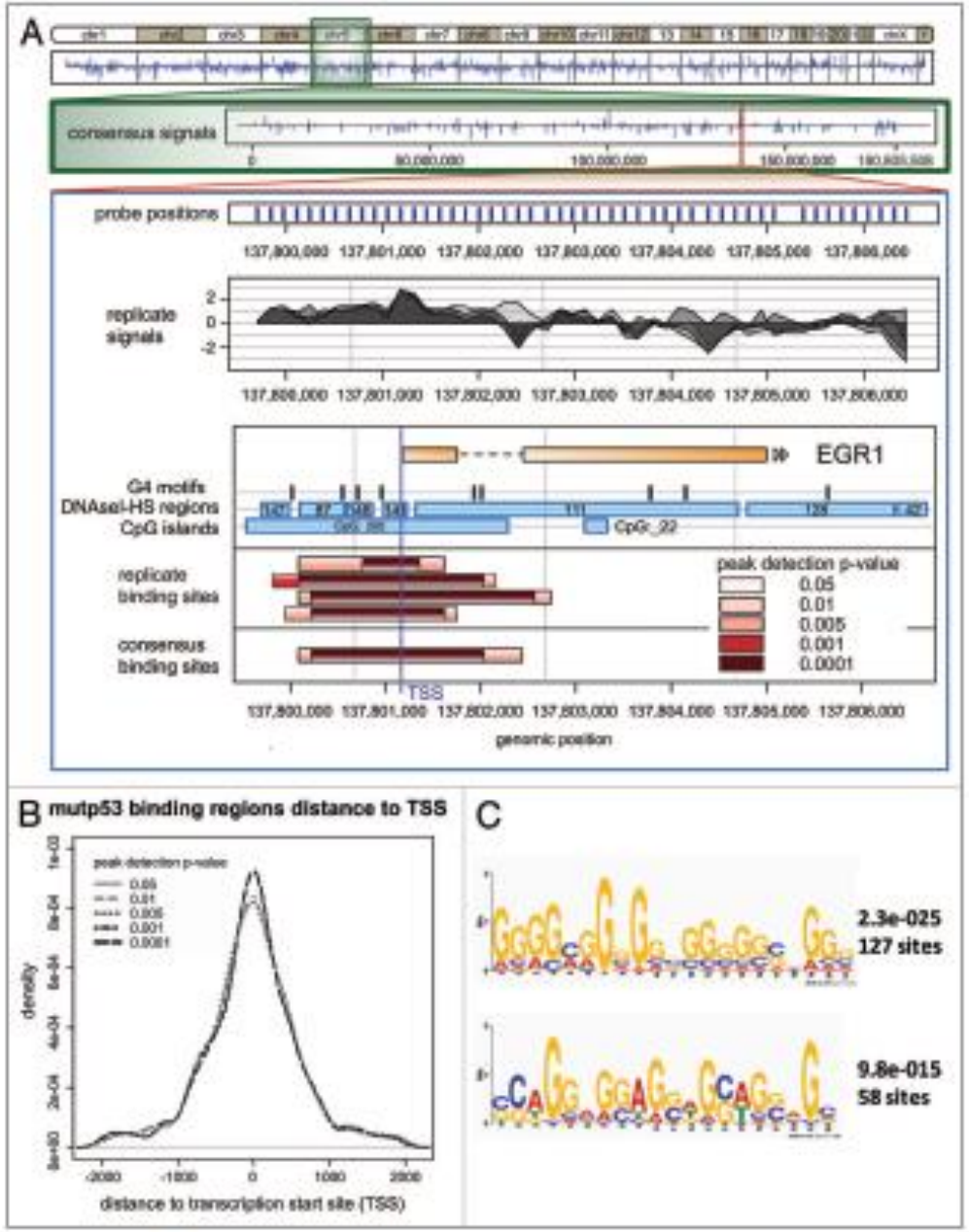


Image credit: Song, C., Zhang, S., and Huang, H. (2015). Choosing a suitable method for the identification of replication origins in microbial genomes. Front. Microbiol.



ChIP- seq
ChIP-cloning
ChIP on ChIP



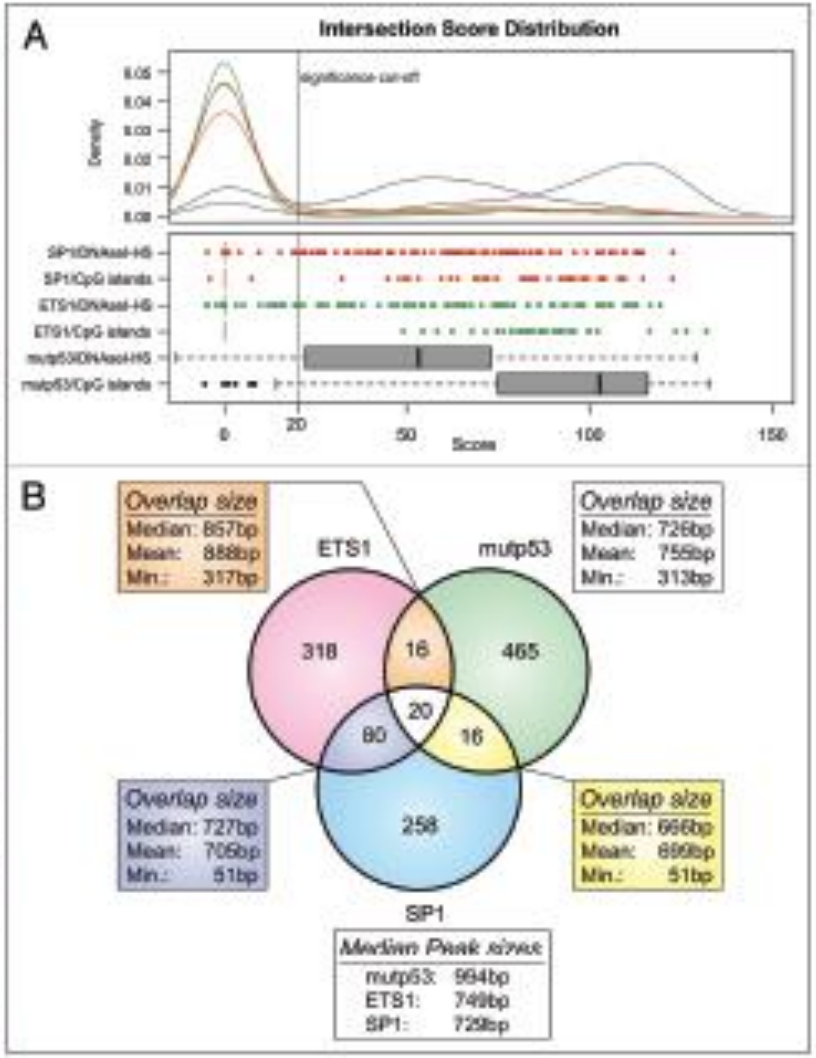


Figure 1. Array-wide analysis of mutp53 binding sites. (A) Consensus signals for the whole genome and for chromosome 5 are shown. The *EGR1* gene is shown as an example of a known mutp53 target gene. The lower panel displays the detected peaks in the EGR1 gene dependent on the selected p-value threshold during peak calling. CpG islands, DNaseI-HS regions and G4 motif locations extracted from the public databases are plotted. (B)

Figure 3. Array-wide analysis of mutp53, SP1 and ETS1 binding sites. (A) Distribution of the scores calculated for the overlap of mutp53, ETS1 and SP1 binding regions with CpG-islands and DNaseI-HS regions. The distribution was used to identify a significance threshold, where peaks with a score >= 20 are assumed to overlap significantly. (B) Venn-diagram displaying the number of overlapping mutp53, ETS1 and SP1 binding regions.

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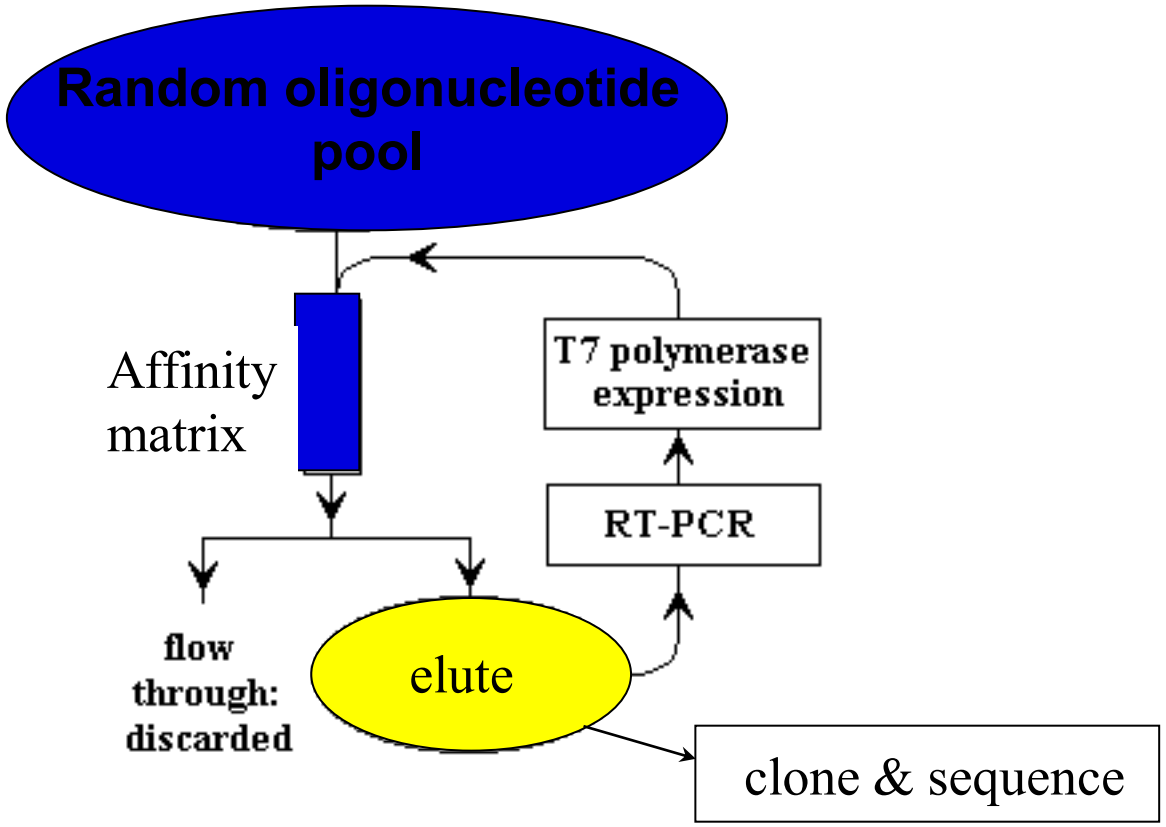
SELEX

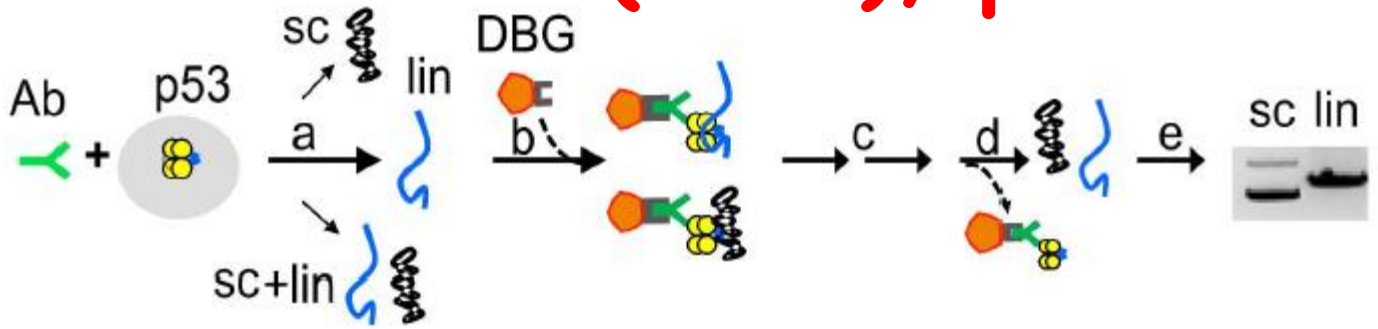
Systematic evolution of ligands by exponential enrichment (**SELEX**), also referred to as *in vitro selection* or *in vitro evolution*, is a combinatorial chemistry technique in molecular biology for producing oligonucleotides of either single-stranded DNA or RNA that specifically bind to a target ligand or ligands. These single-stranded DNA or RNA are commonly referred to as aptamers.[1][2][3] Although SELEX has emerged as the most commonly used name for the procedure, some researchers have referred to it as **SAAB** (selected and amplified binding site) and **CASTing** (cyclic amplification and selection of targets)[4][5] SELEX was first introduced in 1990. In 2015 a special issue was published in the Journal of Molecular Evolution in the honor of quarter century of the SELEX discovery.[6]

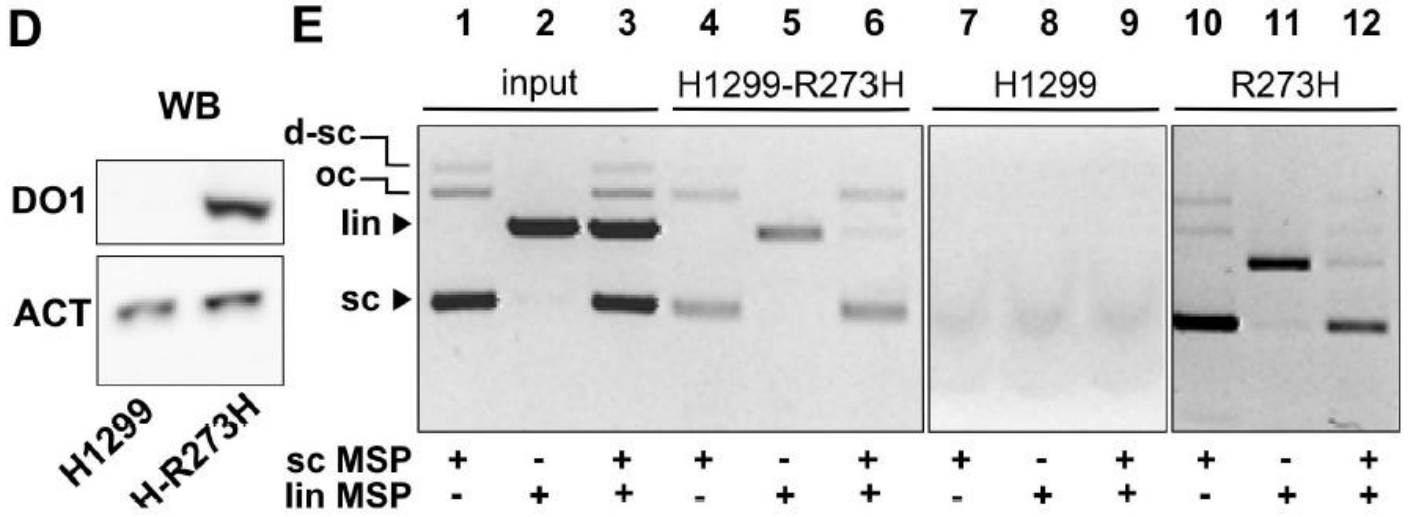


C.Tuerk, L. Gold Systematic evolution of high-affinity RNA ligands of bacteriophage T4 DNA polymerase in vitro. Science 249:505-510 (1990).

Imunoprecipitation-protein -DNA (DNA), pull down

- method of isolating specific proteins from mixtures (lysates, purified...) using antibodies
- antibodies in a complex with their antigens are separated from other molecules using proteins A or G (source of bacteria), which bind immunoglobulins and at the same time are immobilized on a solid surface (BEADS)
- proteins A and G bind to the Fc region of heavy chains
the Fab region is still available for antigen binding
DNA precipitation





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PLOS ONE

Preferential Binding of Hot Spot Mutant p53 Proteins to Supercoiled DNA *In Vitro* and in Cells

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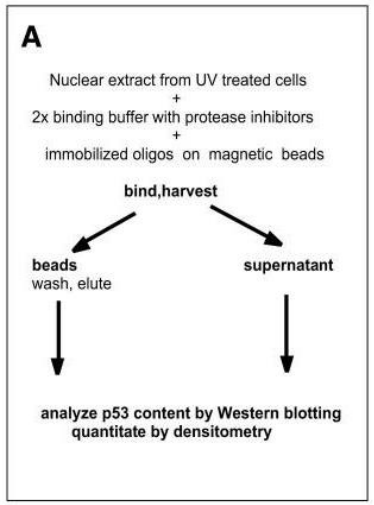
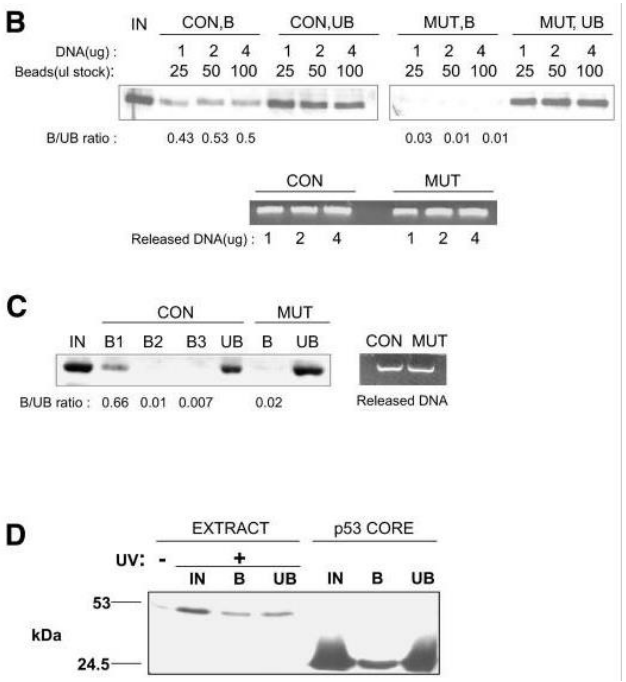
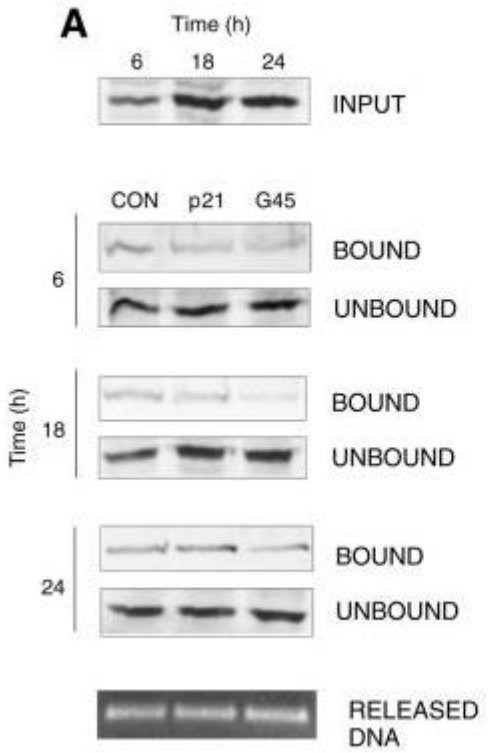
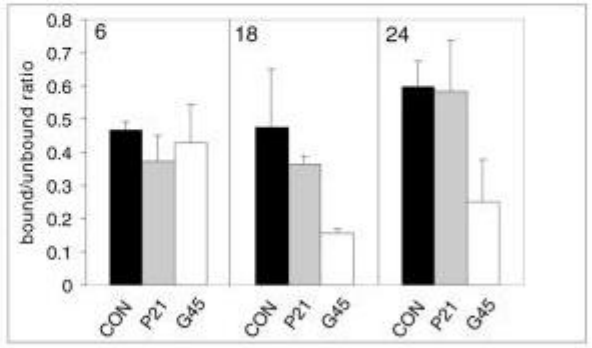
Imunoprecipitation protein–DNA - imobilisation

- method of isolating specific proteins from mixtures (lysates, purified...) by means of DNA-bound to beads
- protein precipitation
- WB detection









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Different systems of affinity tags and their interaction partners used for protein purification

Tab. 1. Různé systémy afinitních značek a jejich interakčních partnerů využívané pro purifikaci proteinů nebo studium protein-proteinových interakcí (afinitní koprecipitaci).

	afinitní značka	sekvence afinitní značky	imobilizovaný interakční partner
Peptidové značky	FLAG	DYKDDDDK	protilátka anti-FLAG
	HA	YPYDVPDYA	protilátka anti-HA
	oligoHis (6-10mer)	HHHHHH(HHHH)	chelát niklu nebo kobaltu
	Myc	EQKLISEEDL	protilátka anti-Myc
	SBP	MDEKTTGWRGGHVVEGLAGELEQLR ARLEHHPQGQREP	streptavidin
	Avi	GLNDIFEAQKIEWHE	streptavidin
	Strep	WSHPQFEK	streptavidin
	V5	GKPIPNPLLGLDST	protilátka anti-V5
Proteinové značky	GST (glutathione S-transferase)		glutathion
	MBP (manose-binding protein)		amylóza

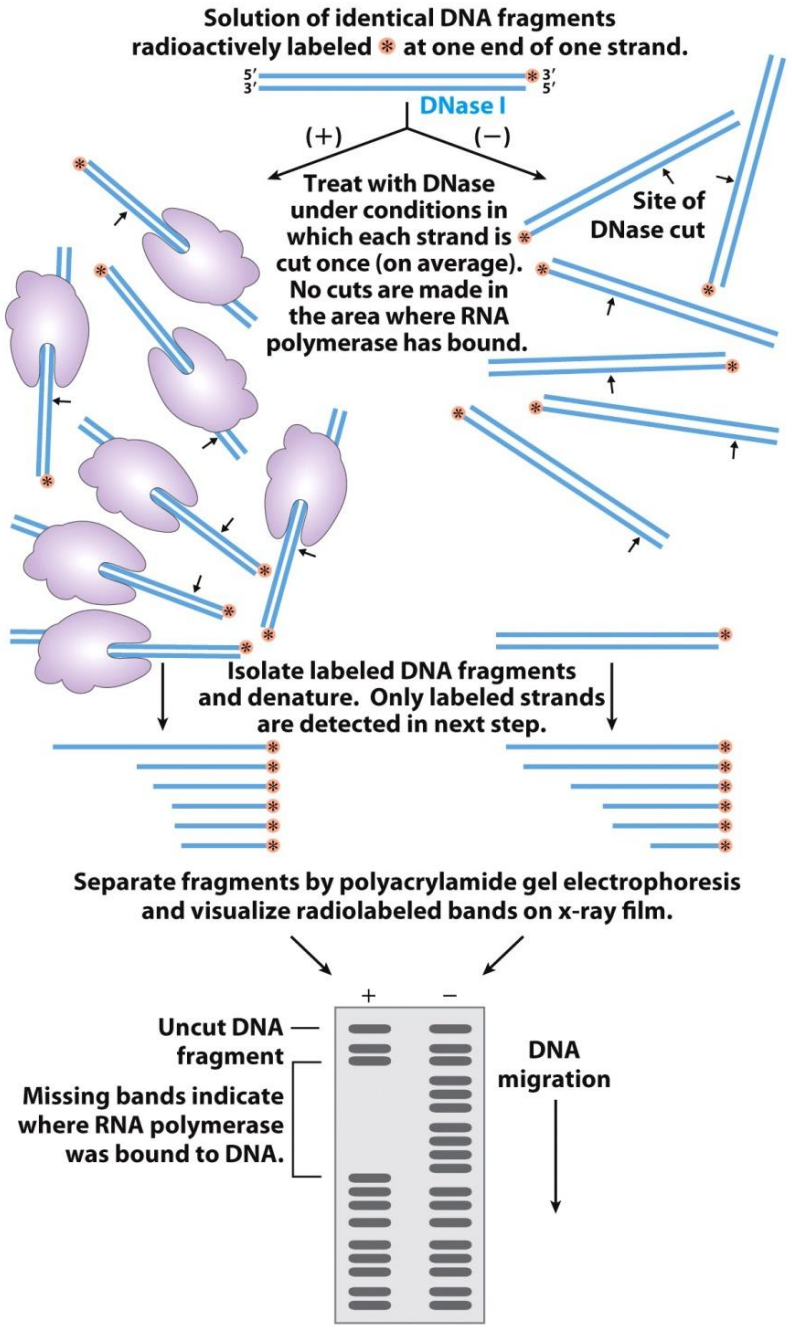
MB2024-DNA protein interactions https://www.sinobiological.com/resource/protein-review/affinity-tag

Protein-DNA Footprinting

"Footprinting" is a technique to identify the DNA-binding site

- Used to identify the target region of interaction within the DNA sequence
- One strand of DNA must be labeled (Radioactive/fluorescent)
- Uses DNase I enzyme or chemical cleavage (piperidine)
- Areas where interaction occurs are protected from cleavage
– missing stripes (bands)

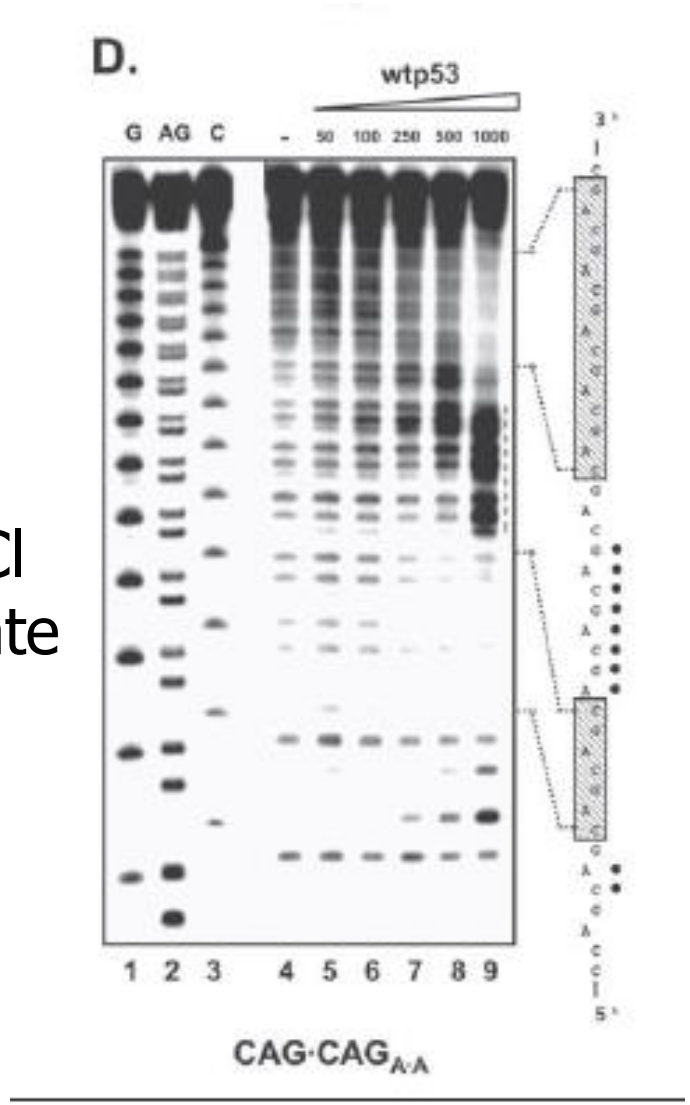
assumption: protein-bound DNA will be protected from chemical cleavage at the binding site.
Isolate the DNA fragment that contains the binding site and "label". Bind the protein to the DNA in one tube; keep the other as a "naked DNA" control
Treat both samples with a chemical or enzymatic agent.
Separate the fragments by gel electrophoresis and visualize the bands on X-ray film or an imaging plate

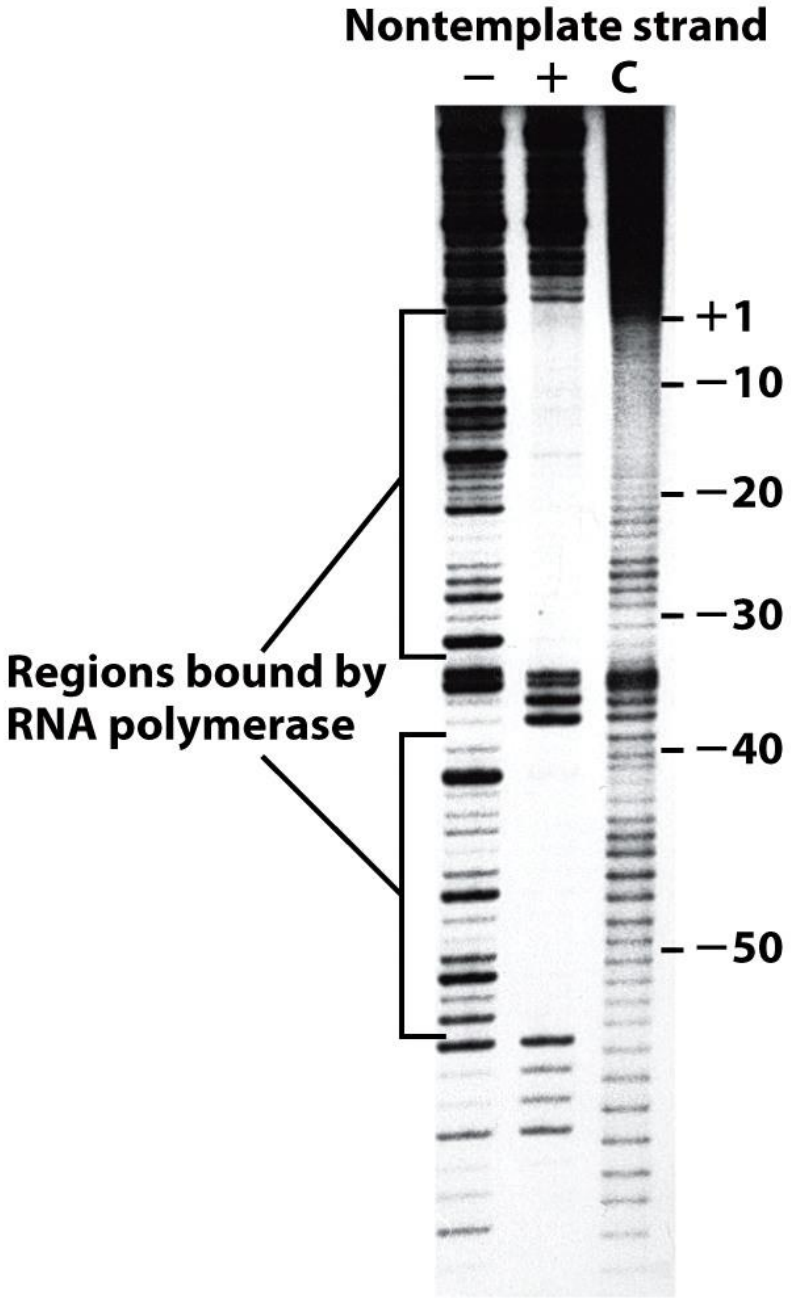


Box 26-1 Figure 1
Lehninger Principles of Biochemistry, Sixth Edition

DNA/RNA footprinting

Chemic cleavage:
AG: Formic acid
CT:Hydrazine
C:Hydrazine+NaCl
G:Dimethylsulphate
-piperidinu

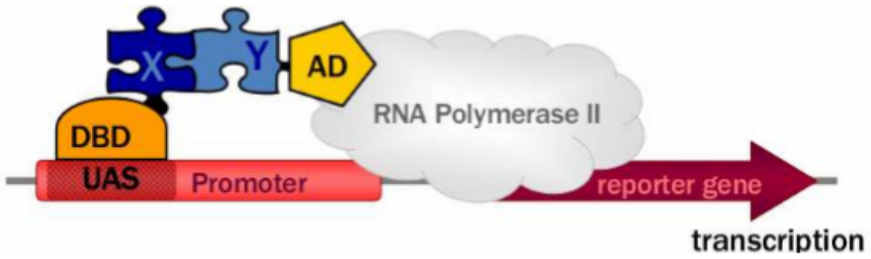




Box 26-1 Figure 2
Lehninger Principles of Biochemistry, Sixth Edition

Yeast two-hybrid systems Y2H

- a molecular biology method commonly used to study interactions between proteins.
- The functioning of Y2H is made possible by the fact that transcription factors have a modular structure and these module-domains can function separately or in fusion with another protein.
- In the most common form of Y2H, the yeast transcription factor Gal4 is split in this way. Its DNA binding domain is fused to one protein ("bait"), usually known, whose binding partners Y2H will search for, while the activation domain of Gal4 is fused to a protein ("prey") that is a potential binding partner of the investigated protein .
- The interaction between "bait" and "prey" restores the original function of Gal4, which subsequently transactivates the relevant reporter genes. Due to its versatility and simplicity, Y2H can be used to rapidly analyze large cDNA libraries encoding proteins fused to the Gal4 DNA binding domain. However, Y2H results must be verified by other methods, as they are usually loaded with a significant error, either false negative or false positive results.
- The yeast two-hybrid system, its possible uses, variations and limitations have recently been described in great detail (Brückner et al. 2009).



Obrázek 2:
Kvasinkový dvouhybridní systém

studovaný protein (X), je fúzován s DNA vazebnou doménou, (DBD) pocházející nejčastěji z Gal4, která se váže do aktivační oblasti v blízkosti promotoru reportéru (UAS, *upstream activating sequence*), zatímco potenciální vazebný partner (Y) je fúzován s aktivační doménou (AD), obvykle z Gal4. Pokud spolu oba hybridní proteiny interagují, funkce původně rozděleného transkripčního faktoru se obnoví, což vyvolá transkripci reportérového genu RNA polymerázou II. Převzato z (Brückner *et al.* 2009).

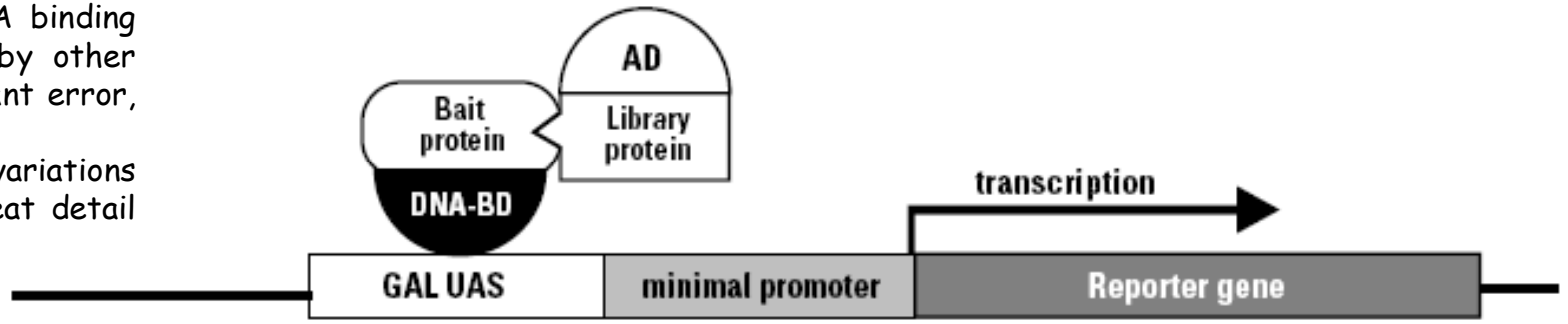


Figure 2. The two-hybrid principle. The DNA-BD is amino acids 1–147 of the yeast GAL4 protein, which binds to the GAL UAS upstream of the reporter genes. The AD is amino acids 768–881 of the GAL4 protein and functions as a transcriptional activator.

Report gene:
lacZ (B-galaktosidase)
Luciferase
GFP

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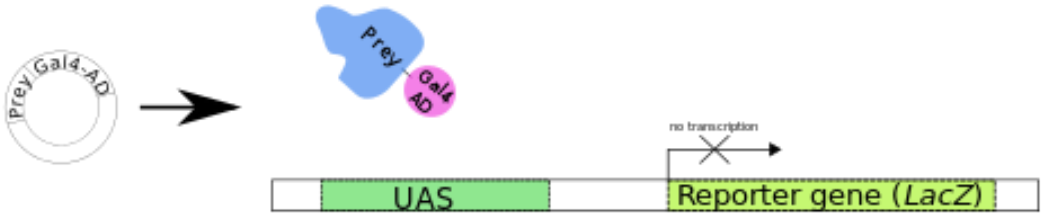
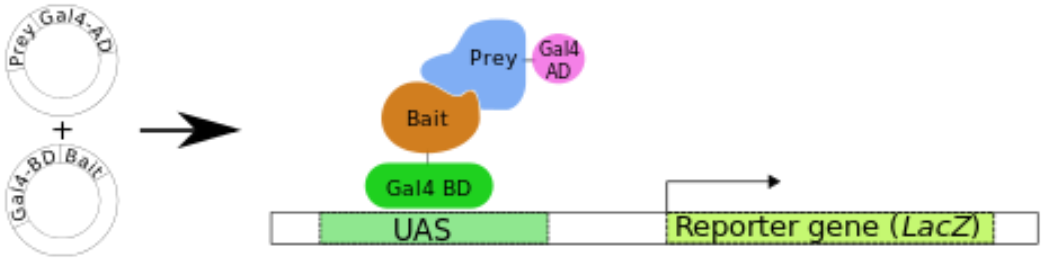
A. Regular transcription of the reporter gene

B. One fusion protein only (Gal4-BD + Bait) - no transcription



C. One fusion protein only (Gal4-AD + Prey) - no transcription



D. Two fusion proteins with interacting Bait and Prey

V nejběžnější podobě Y2H je takto rozdělen kvasinkový **transkripční faktor Gal4**. Jeho **DNA vazebná doména (BD)** je fúzována s **jedním proteinem (bait – „návnada")**, obvykle známým, jehož vazebné partnery bude Y2H vyhledávat, zatímco **aktivační doména (AD) Gal4 je fúzována s proteinem (prey – „kořist")**, který je potenciální vazebný partner zkoumaného proteinu.

Aplikace/Využití:

- protein-protein interakce
- DNA-protein interakce
- Analýza genové exprese- regulace
- reporterový test (DNA vazebné elementy, DNA regulační elementy)

Geny nebo markery reportéra poskytují vhodný prostředek identifikovat a analyzovat regulační prvky genů.
Systém reportérů měří transkripční činnost (interakce cis-prvků na předkladatele s předkladatelem trans-působící faktory).

https://cs.wikipedia.org/wiki/Dvouhybridový_systém

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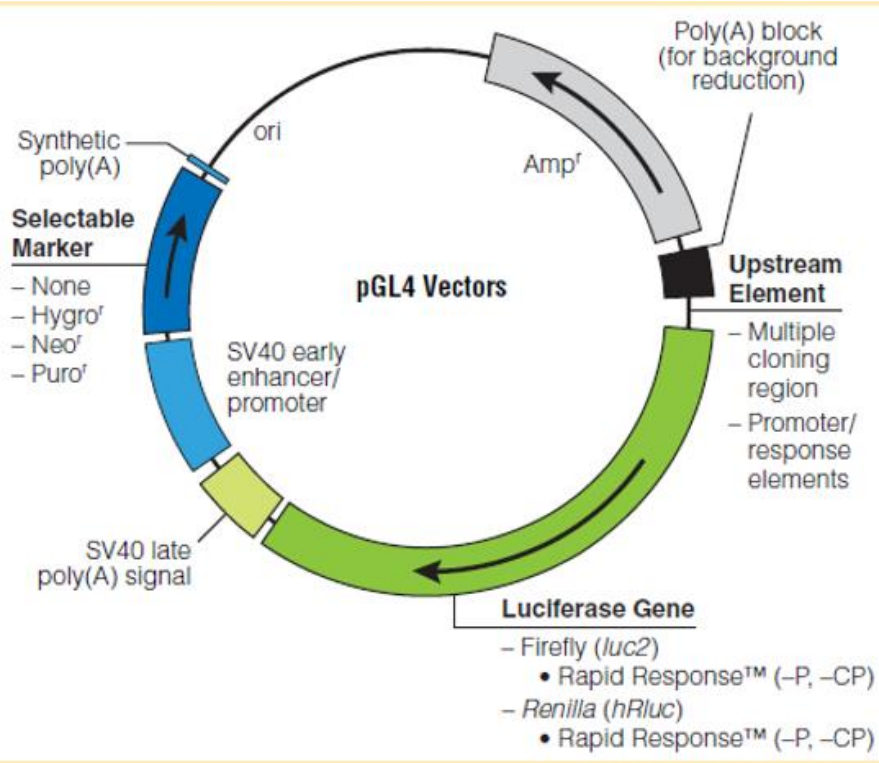
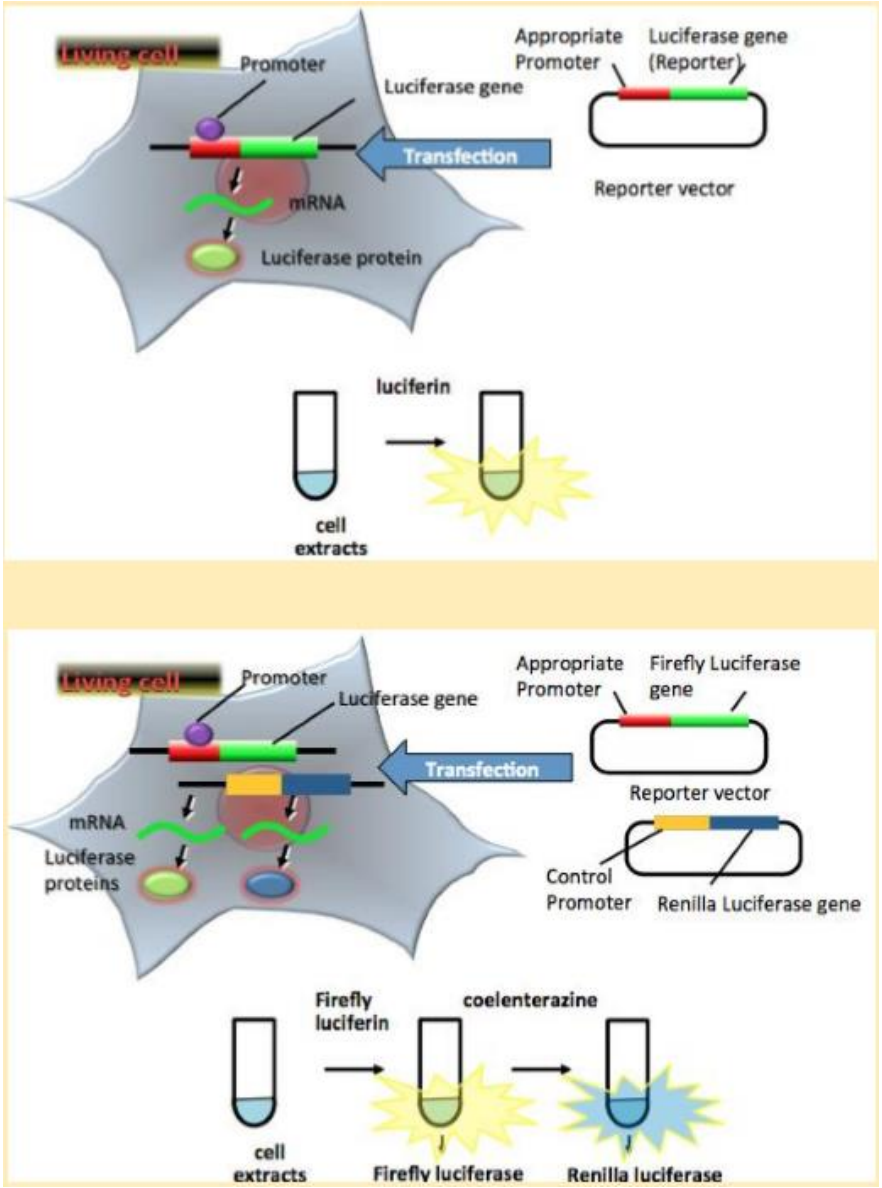
Dual-Luciferase® Reporter (DLR™) Assay





The Dual-Luciferase® Reporter (DLR™) Assay System(a–c) provides an efficient means of performing dual-reporter assays. In the DLR™ Assay, the activities of **firefly (Photinus pyralis) and Renilla (Renilla reniformis, also known as sea pansy)** luciferases are measured sequentially from a single sample. The firefly luciferase reporter is measured first by adding Luciferase Assay Reagent II (LAR II) to generate a stabilized luminescent signal. After quantifying the firefly luminescence, this reaction is quenched, and the Renilla luciferase reaction is simultaneously initiated by adding Stop & Glo® Reagent to the same tube.

http://photobiology.info/Ohmiya.html

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Luciferase Reporter Assay

– The Dual-Luciferase® Reporter (DLR™) Assay System(a–c) provides an efficient means of performing dual-reporter assays. In the DLR™ Assay, the activities of **firefly (Photinus pyralis) and Renilla (Renilla reniformis, also known as sea pansy)** luciferases are measured sequentially from a single sample. The firefly luciferase reporter is measured first by adding Luciferase Assay Reagent II (LAR II) to generate a stabilized luminescent signal. After quantifying the firefly luminescence, this reaction is quenched, and the Renilla luciferase reaction is simultaneously initiated by adding Stop & Glo® Reagent to the same tube.

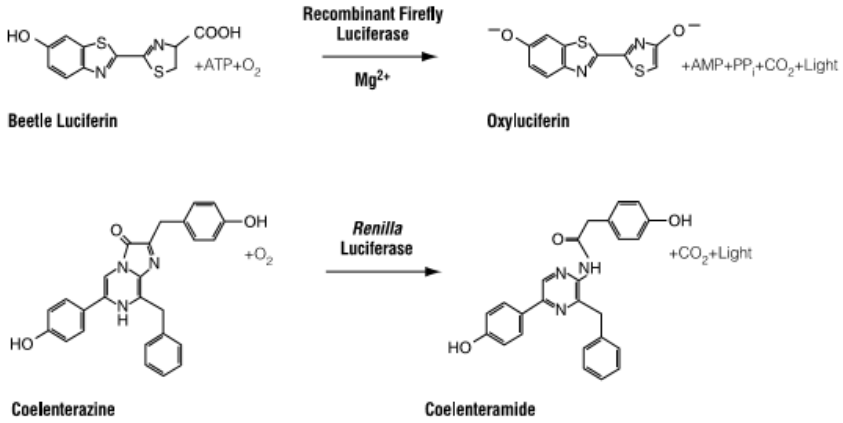
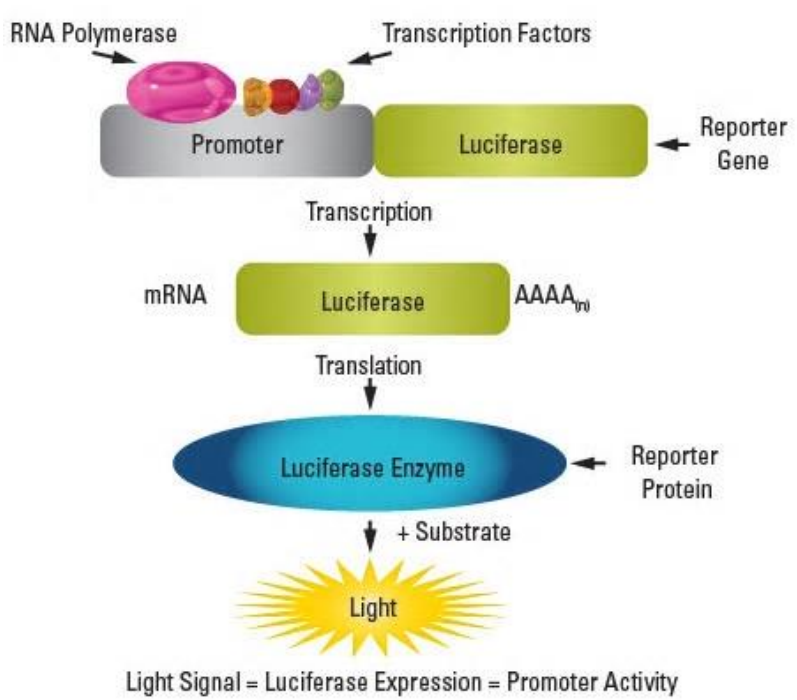


Figure 1. Bioluminescent reactions catalyzed by firefly and *Renilla* luciferases.



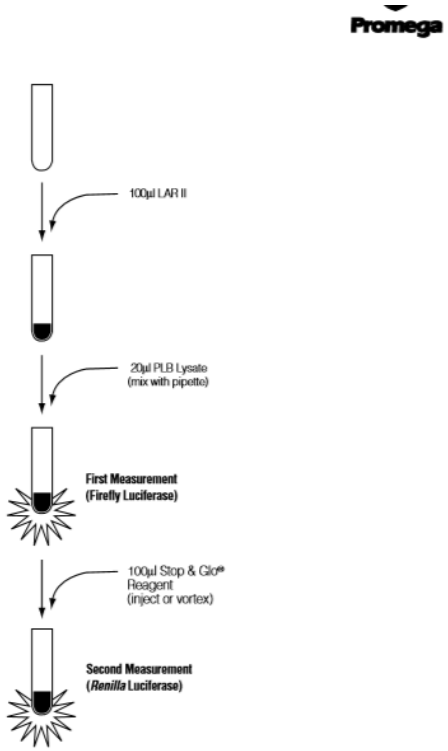


Figure 6. Format of the DLR™ Assay using a manual luminometer or a luminometer equipped with one reagent injector. If the instrument is equipped with two injectors, it may be preferable to predispense the lysate into luminometer tubes, followed by sequential auto-injection of the LAR II and Stop & Glo® Reagents.

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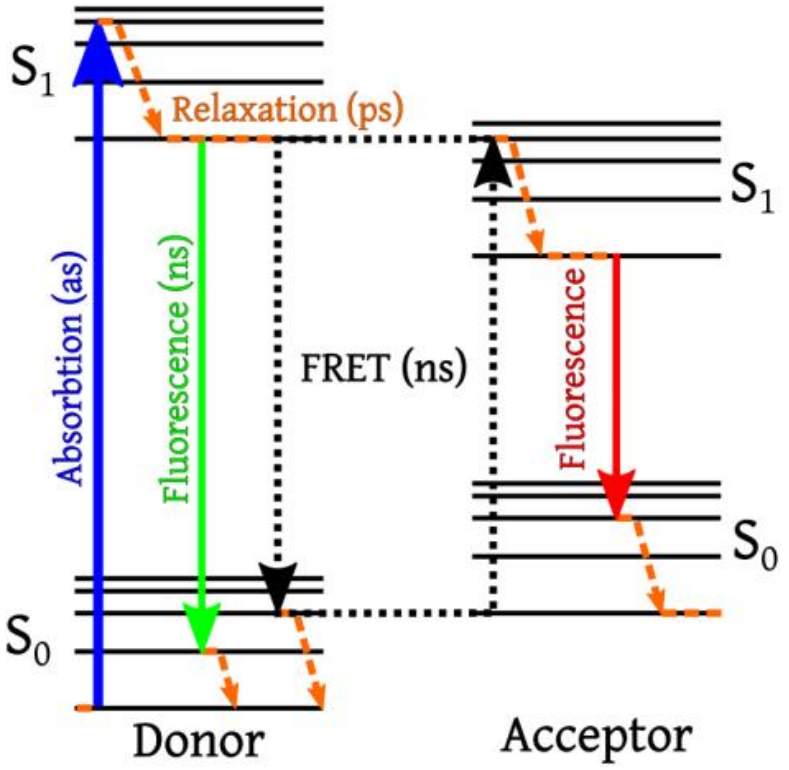
FRET Förster/fluorescence resonance energy transfer

Fluorescence / Förster resonance energy transfer

Measurements of FRET efficiency can be used to determine if two fluorophores are within a certain distance of each other.[5] Such measurements are used as a research tool in fields including biology and chemistry. is a mechanism describing energy transfer between two light-sensitive molecules (chromophores).[1] A donor chromophore, initially in its electronic excited state, may transfer energy to an acceptor chromophore through nonradiative dipole–dipole coupling

FRET Pair Fluorescent Proteins

Laser	Donor	Acceptor	Donor Ex Acceptor Em
Violet	CFP	YFP	405/526
Violet	Cerulean FP	YFP	405/526
Argon	GFP	YFP	488/526
Argon	GFP	mRFP	488/579



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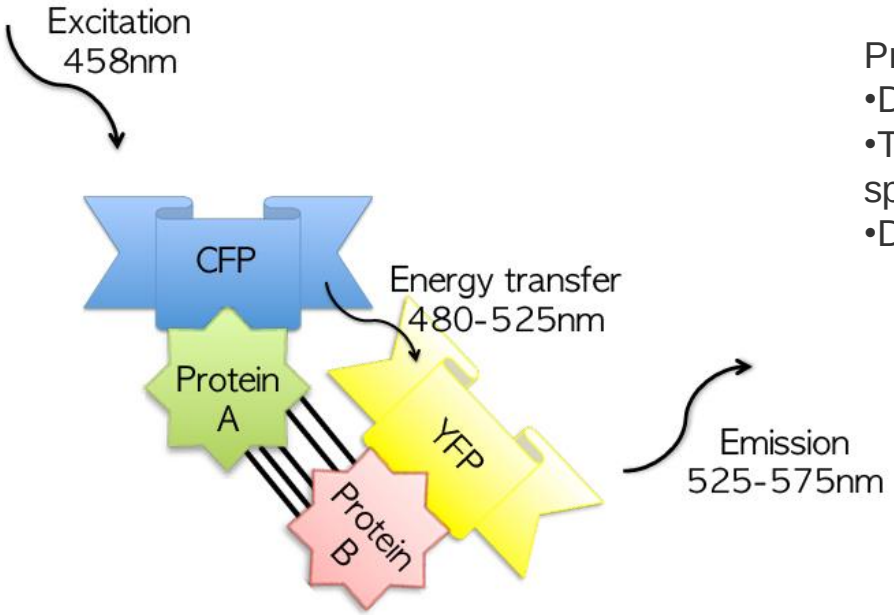
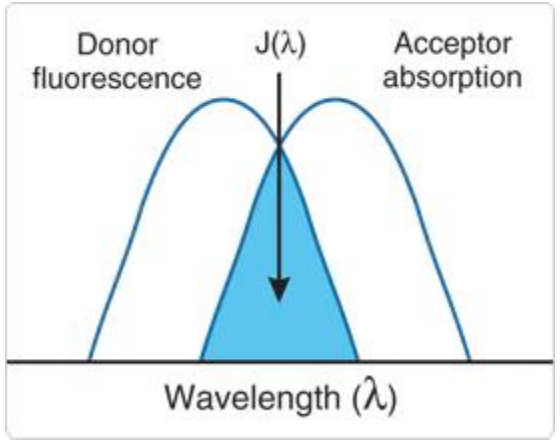
FRET Förster/fluorescence resonance energy transfer



Primary Conditions for FRET
•Donor and acceptor molecules must be in close proximity (typically 10–100 Å).
•The absorption spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor (**Figure 1**).
•Donor and acceptor transition dipole orientations must be approximately parallel.



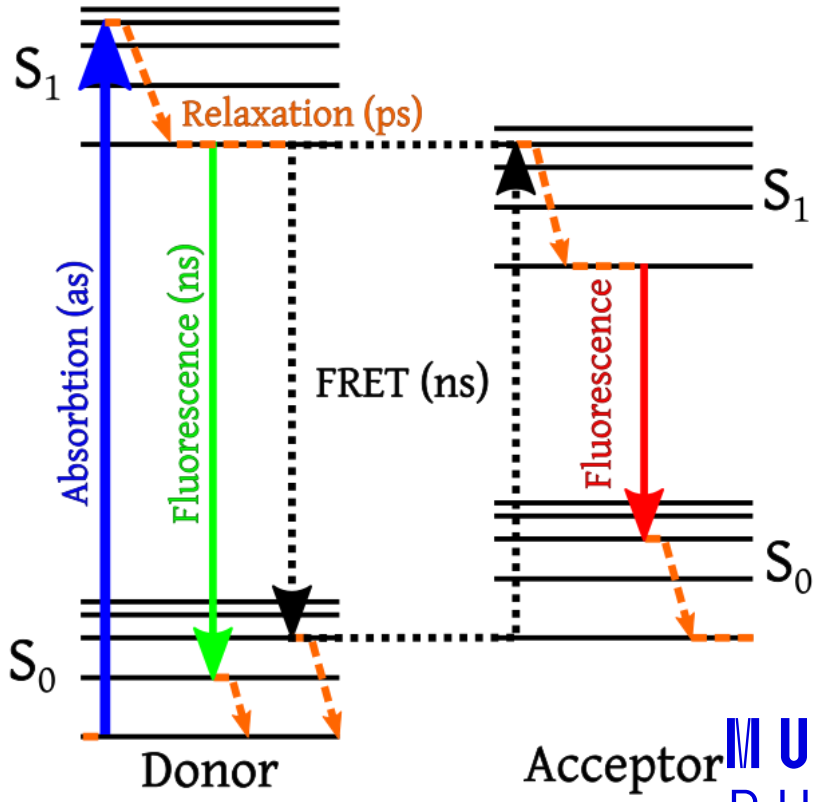


Table 1. Typical Values of R_0

Donor	Acceptor	R_0 (Å)
Fluorescein	Tetramethylrhodamine	55
IAEDANS	Fluorescein	46
EDANS	Dabcyl	33
Fluorescein	Fluorescein	44
BODIPY FL	BODIPY FL	57
Fluorescein	QSY 7 and QSY 9 dyes	61

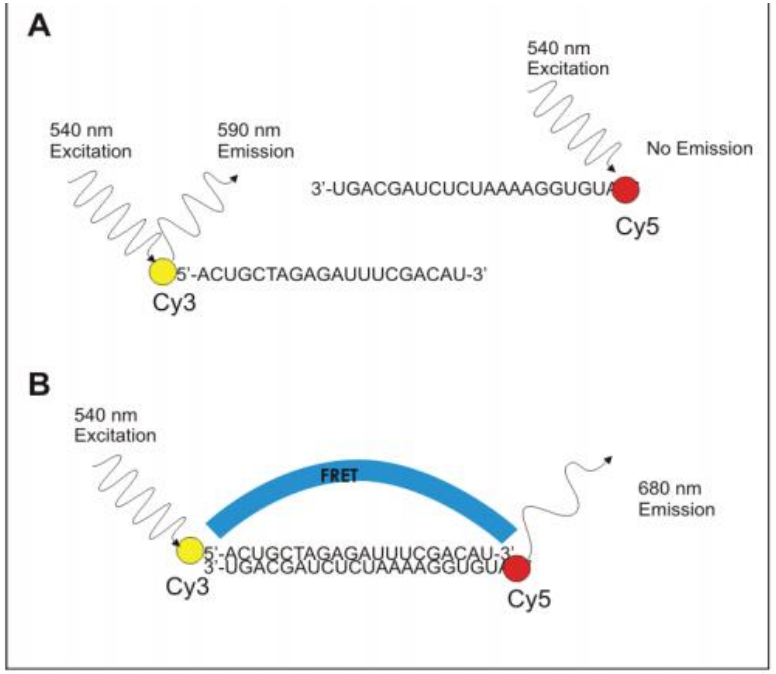


Figure 4. Schematic representation of FRET occurring between Cy3 and Cy5 fluorescent moieties when labeled oligonucleotides are annealed.

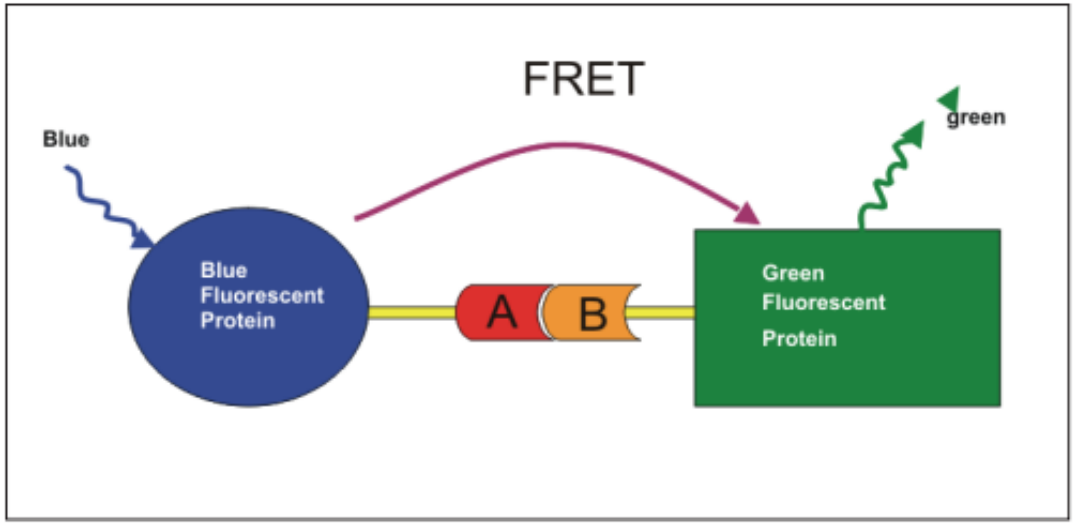
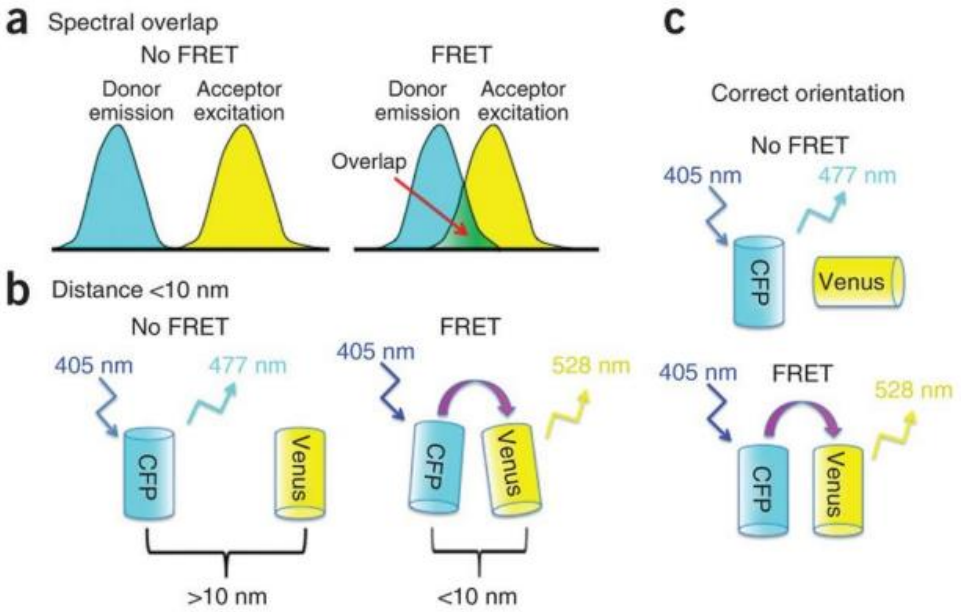


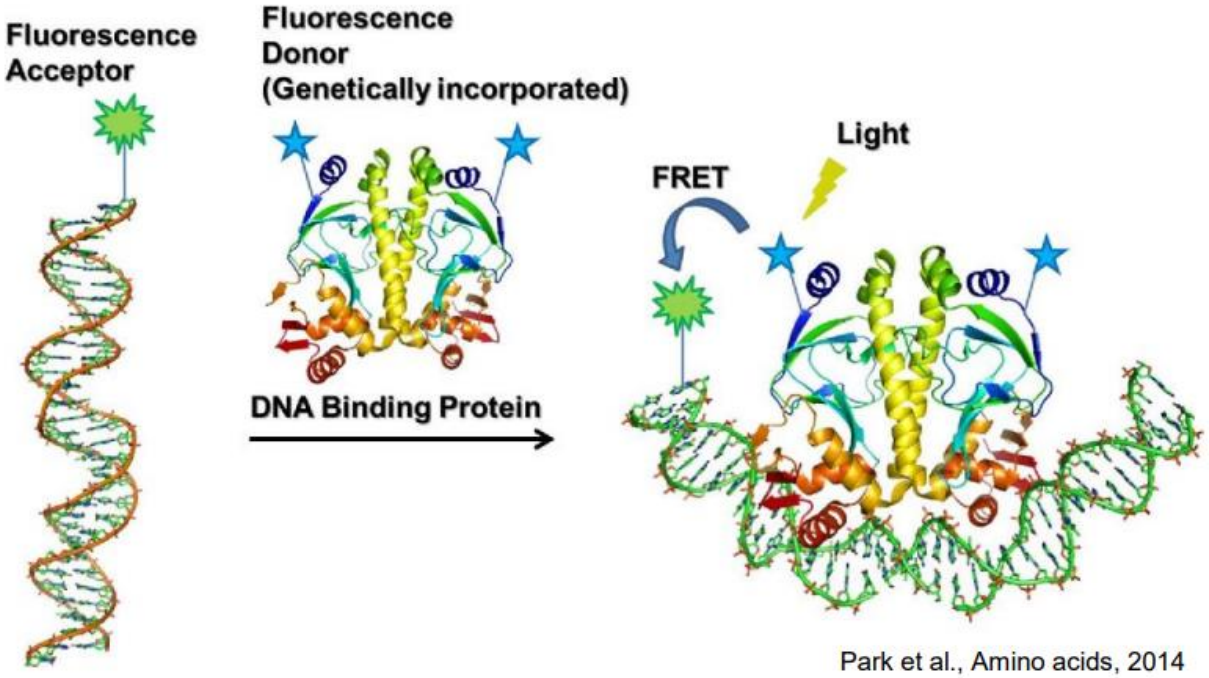
Figure 3. **Schematic representation of the interaction of two different fluorescent protein chimeras.** Protein-protein interactions between proteins labeled A and B bring Blue fluorescent protein and Green fluorescent proteins in close enough proximity to allow for FRET to occur. In this example, excitation of blue fluorescent protein results in the emission of fluorescence by Green fluorescent protein.

FRET

Fluorescence / Förster resonance energy transfer



https://www.photometrics.com/learn/physics-and-biophysics/fret



Park et al., Amino acids, 2014

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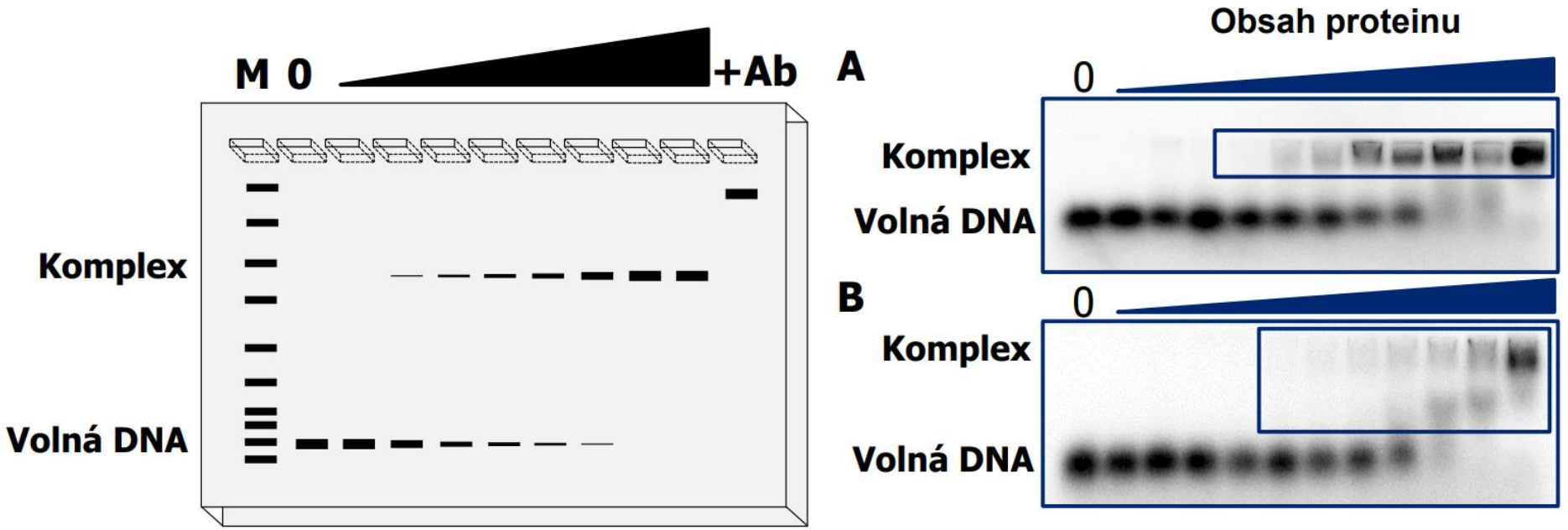
Electro-Mobility Shift Assay (EMSA)



Determination of protein-DNA interaction affinity
- The interaction takes place in vitro
- Necessity of DNA labeling (Radioactivity/fluorescence)
- Separation by gel electrophoresis (PA, Agarose)
- We monitor the retardation (shift) of the migration of the DNA-protein complex in the electric field
- The migration of a molecule (complex) in an electric field depends on its size and charge

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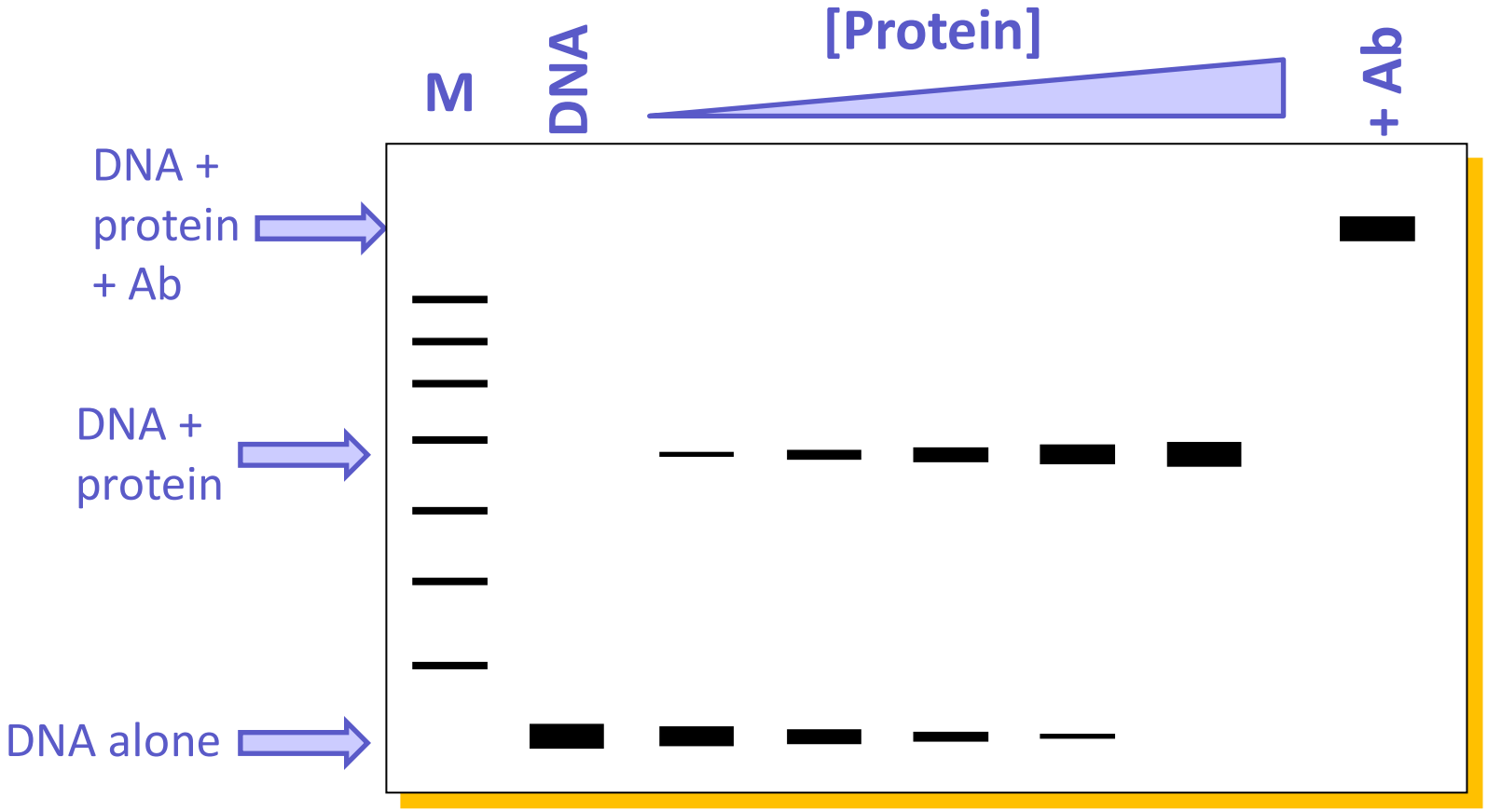
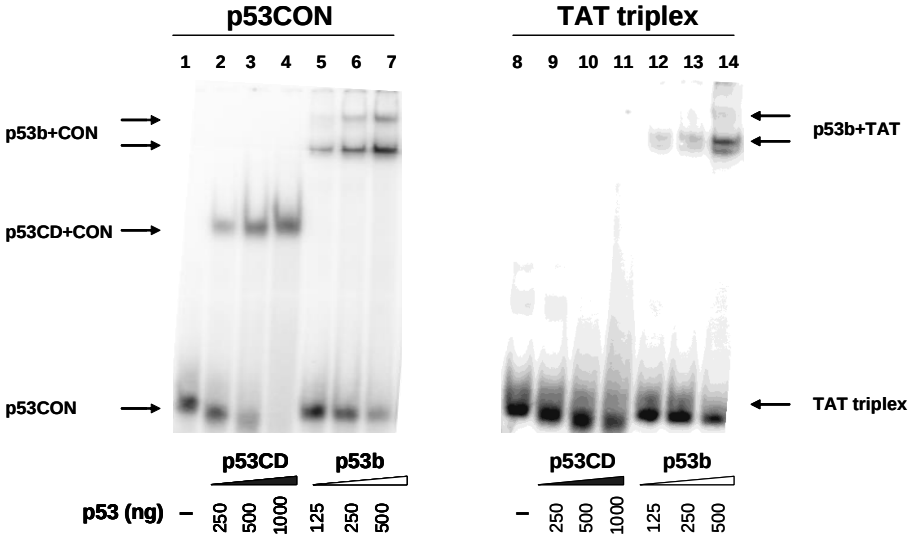
TEST

EMSA ("Gel Shift" Assay)

- Electrophoretic Mobility Shift Assay (EMSA) or "gel shift" can provide information about protein-NA interactions



CD x C-terminus



Poměrně přímočará technika, ale poskytuje pouze přesvědčivá data pro interakce s vysokou afinitou (typicky <µM)

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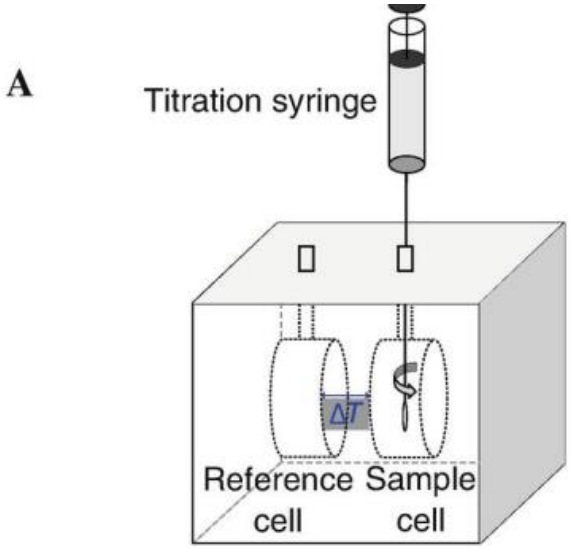
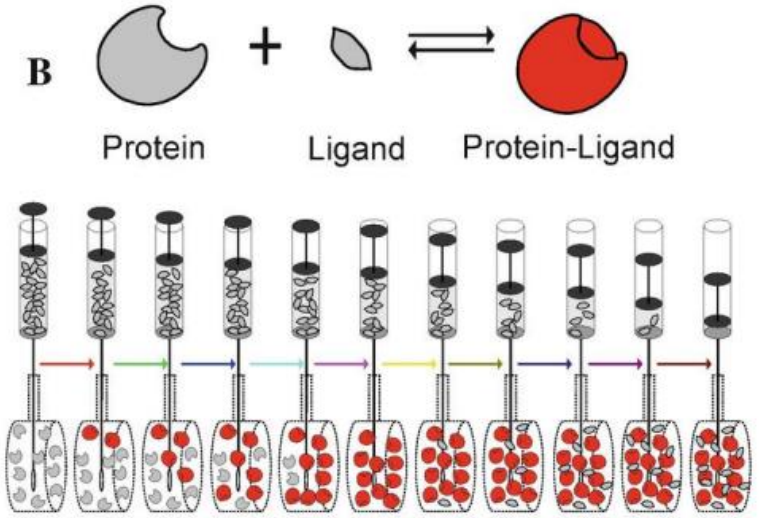
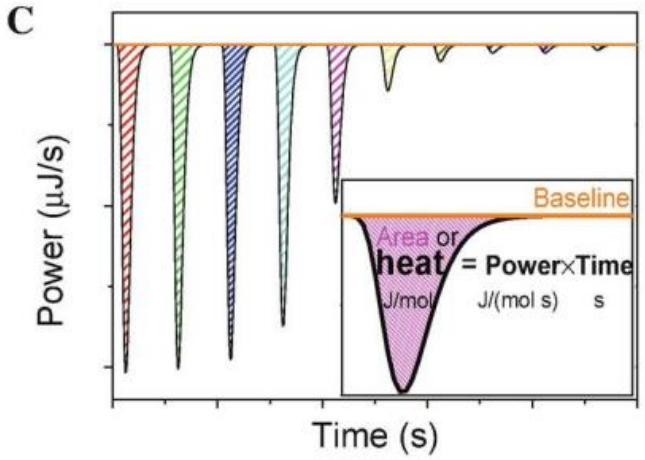
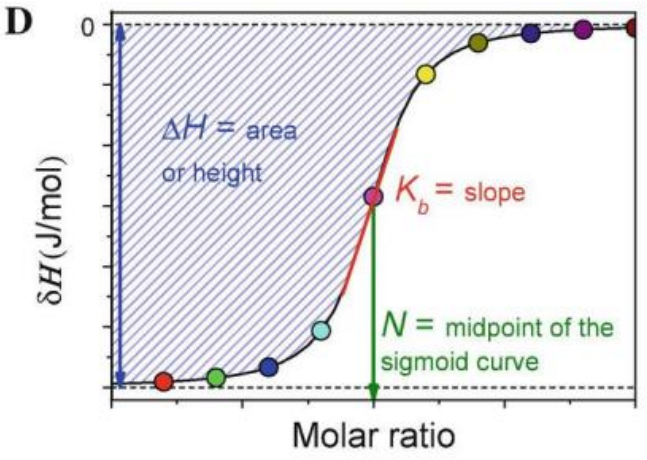
ITC









$$\Delta G = \Delta H - T\Delta S$$

$$\Delta G = RT\ln K_d$$

ΔG- *Gibbs Free Energy, or "available energy"*
ΔH- *Enthalpy change*
T- *Temperature in Kelvin*
ΔS- *Entropy change*
R- *Gas constant, 8.314JK^{-1}mol^{-1}*
K$_d$- *Dissociation rate*

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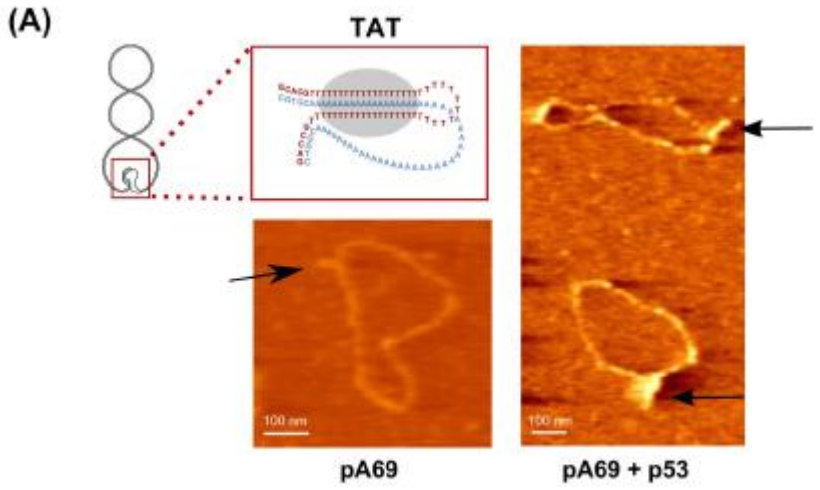
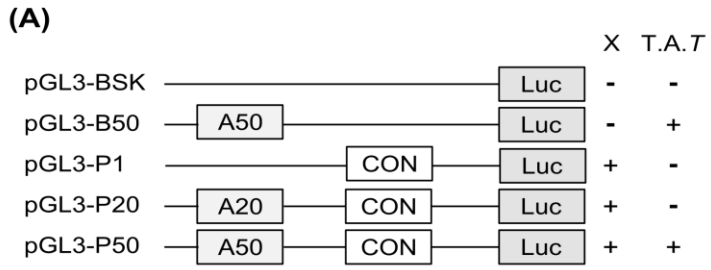
RESEARCH ARTICLE

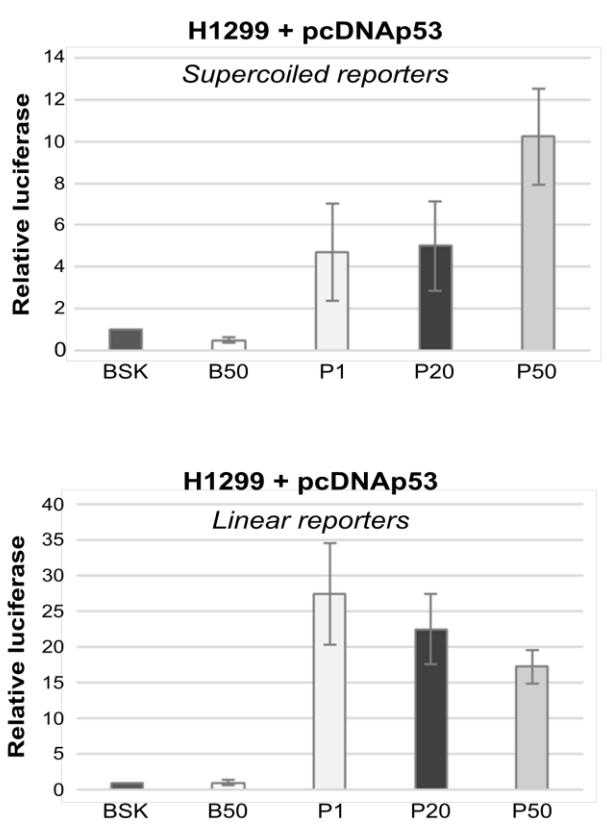
p53 Specifically Binds Triplex DNA *In Vitro* and in Cells

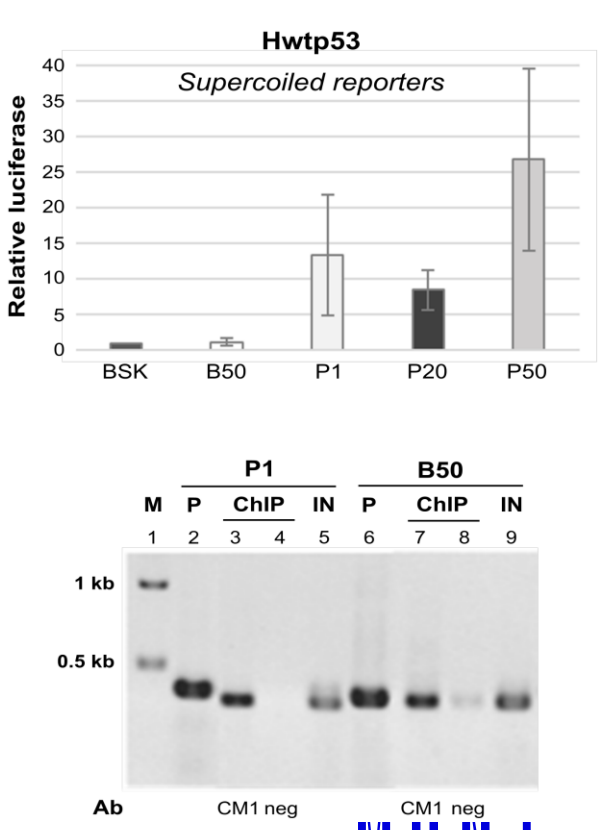
Marie Brázdová[1]*, Vlastimil Tichý[1], Robert Helma[1], Pavla Bažantová[1], Alena Polá Aneta Krejčí[2], Marek Petr[1], Lucie Navrátilová[1], Olga Tichá[1], Karel Nejedlý[1], Martin L. Bennink[3], Vinod Subramaniam[3], Zuzana Bábková[2], Tomáš Martínek[4], Matej Le Matej Adámik[1]

1 Department of Biophysical Chemistry and Molecular Oncology, Institute of Biophysics, Academy Sciences of the Czech Republic v.v.i., Brno, Czech Republic, 2 Department of Molecular Biology a Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Brno, Brno, Czech Republic, 3 Biophysical Engineering Group, Faculty of Science and Technology University of Twente, Enschede, The Netherlands, 4 Department of Computer Systems, Faculty of Information Technology, Brno University of Technology, Brno, Czech Republic, 5 Department of In Technologies, Faculty of Informatics, Masaryk University, Brno, Czech Republic









NMR

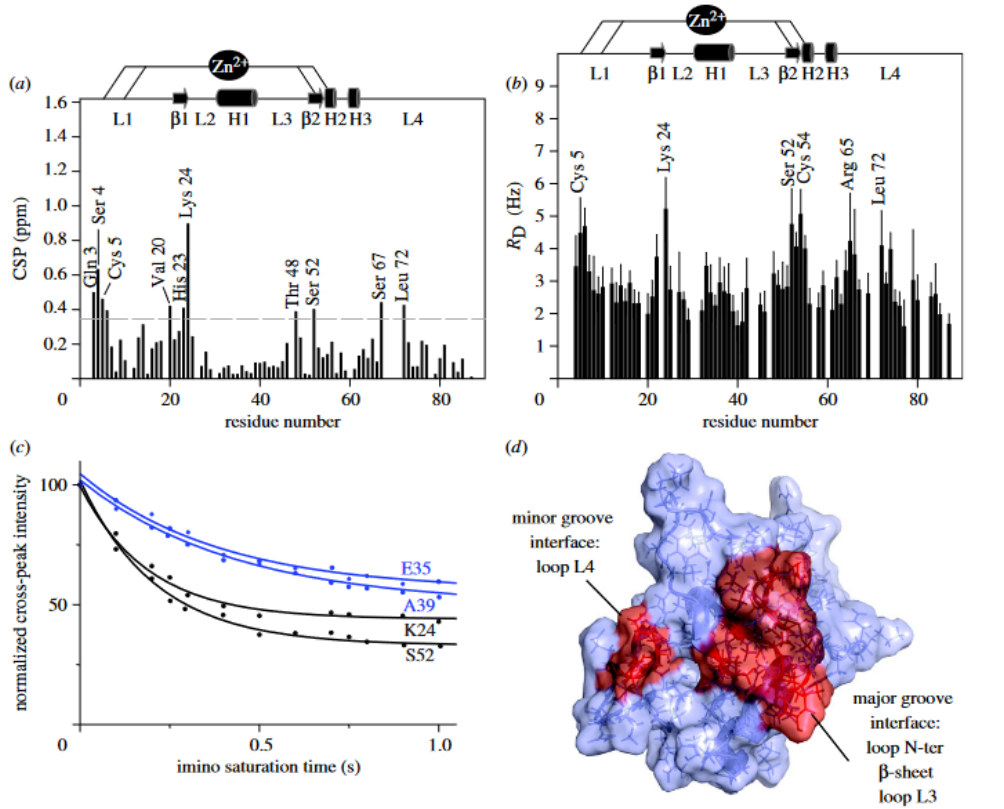
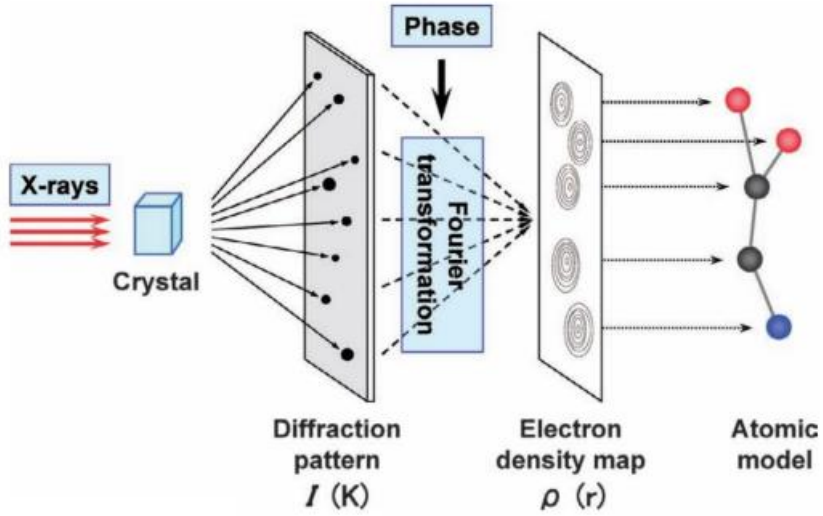
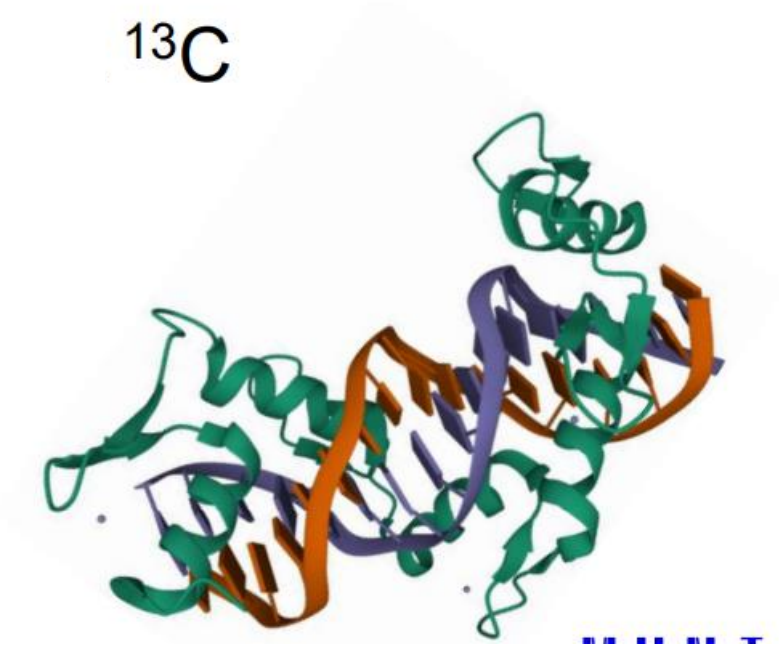


Figure 1. Interaction surface mapping by combining chemical shift perturbation (CSP) and imino cross saturation on the THAP domain of hTHAP1. (*a*) Histogram of the normalized CSP observed upon DNA binding as a function of the residue number. (*b*) Imino cross-saturation rates (R_D) as a function of residue number. (*c*) Examples of experimental points and fitted curves of the imino cross-saturation data. Experimental points and fitted curves are coloured in blue for the α-helical residues (away from DNA) and in black for β-sheet residues (close to DNA). (*d*) Mapping of the interaction surface on the solution structure of the THAP domain of hTHAP1.



^{13}C



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Summary: gene expression analysis

- study of transcription (mRNA-northern transfer, RT-PCR, IN SITU hybridization, primer extension)
comparison of transcriptomes (RT-PCR, siRNA, DNA microarrays, ..)
Analysis of promoters and protein-DNA interactions (reporter genes, promoter localization, identification of promoter regulatory regions-elements, footprinting, EMSA...)
Analysis of translation (proteins-WB, ChIP, IP)