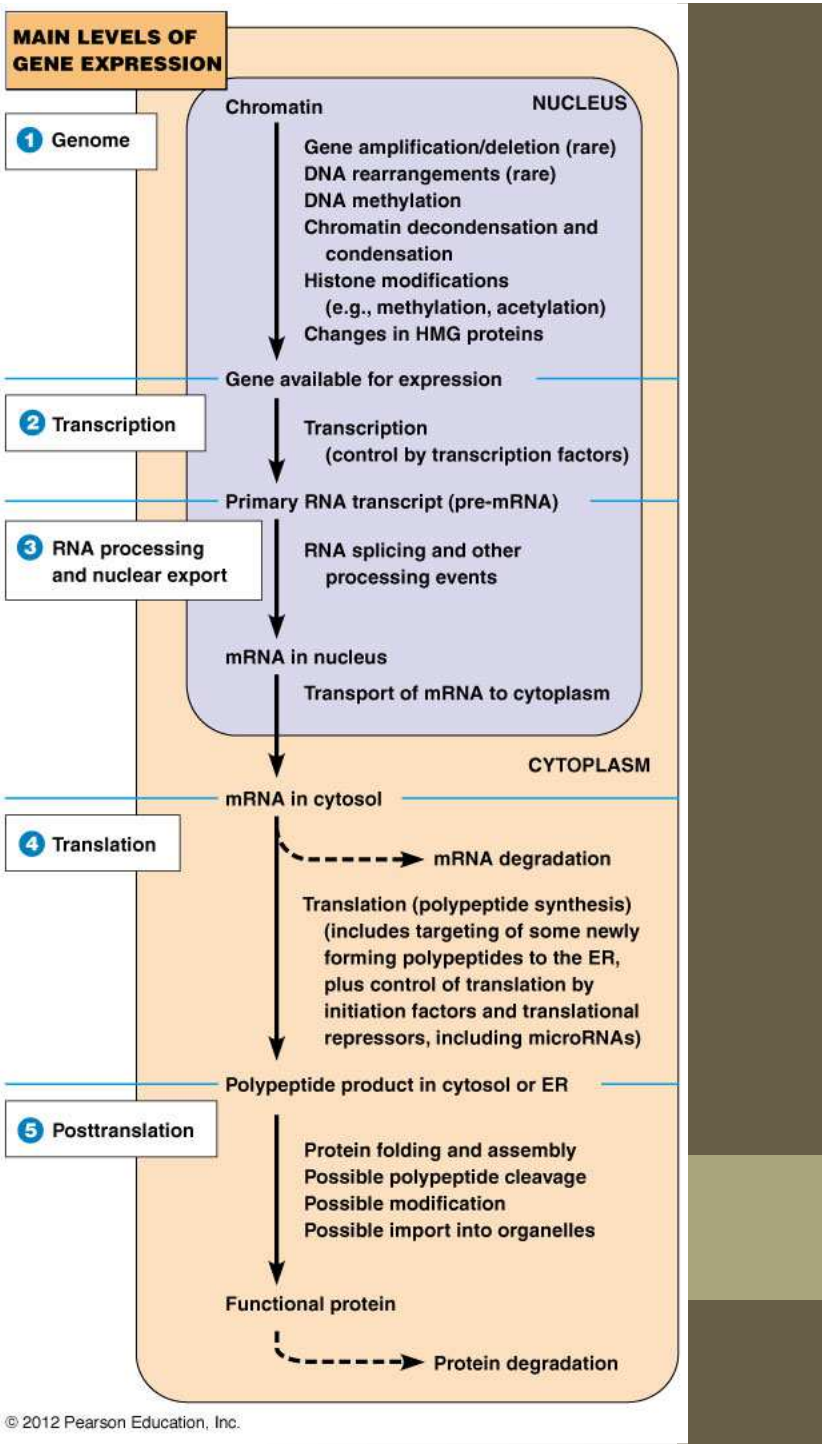
Macromolecole Biologiche, 02-10-2015

TRANSCRIPTION CONTROL ELEMENTS AND METHODS OF PROMOTER ANALYSIS

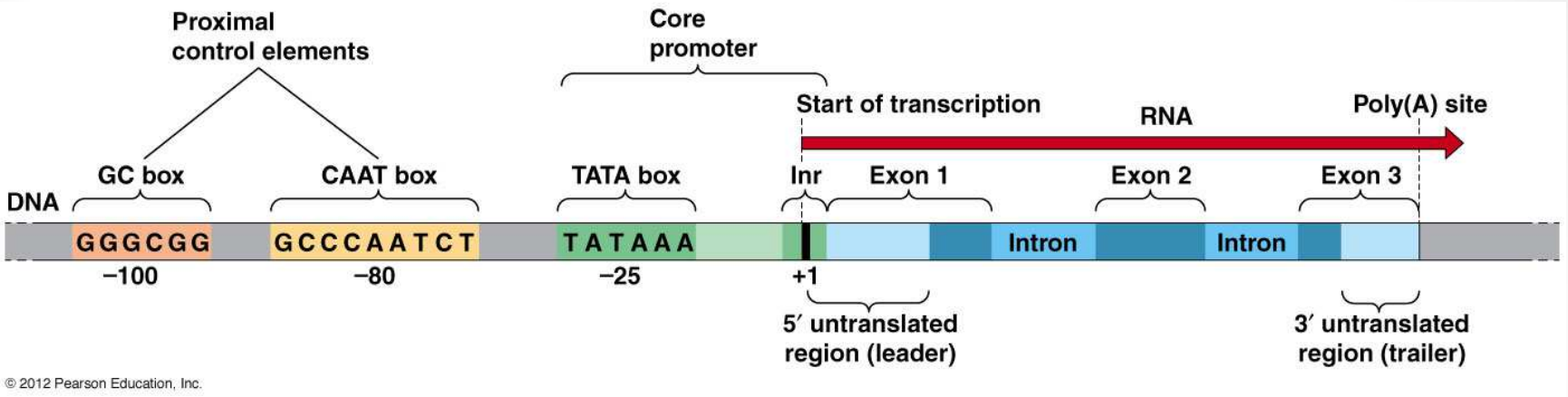
Control of Eukaryotic Gene Regulation

Gene expression can be regulated by events that occur at many levels.

1) **Genome**
2) **Transcription**
3) **Processing** (and nuclear export) of RNA.
4) **Translation** (and targeting) of protein.
5) **Post-translational events** (es.folding)



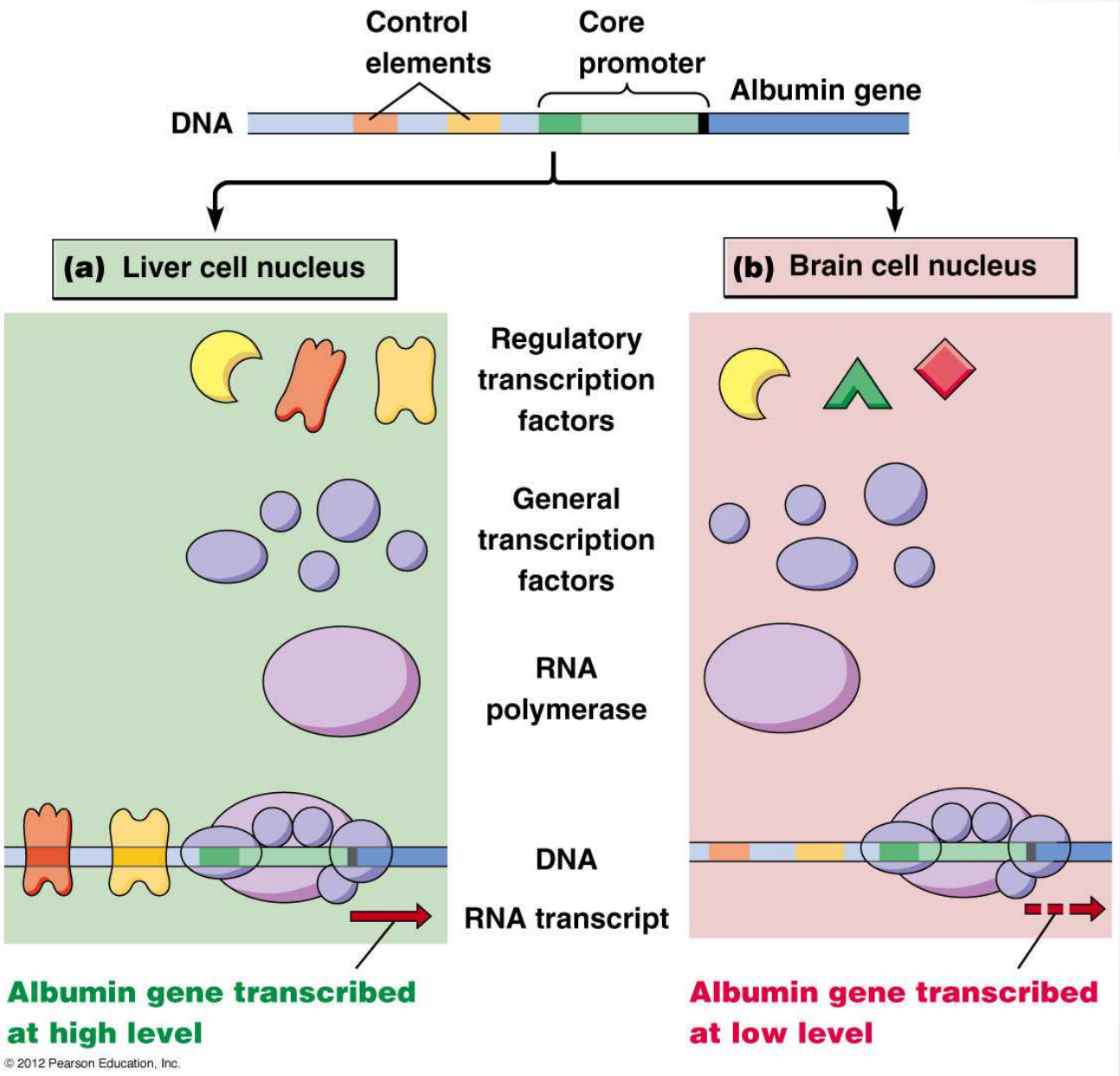
Overview of Eukaryotic Promoters



The promoter of a eukaryotic gene can be defined as a sequence that sets the transcription start site for RNA polymerase. Strong RNA Pol II promoters contain an A/T rich sequence known as the TATA box located 26-31 bp upstream of the start site.

Other genes have alternative sequence elements known as initiators (Inr) which also serve as promoters that set the RNA Pol II start site. Finally, CG-rich repeat sequences (CpG islands) are used by RNA Pol II as promoters in 60-70% of genes.

Different Regulation in Different Tissues



IDENTIFICATION OF PROMOTER REGIONS

How to proceed?

IDENTIFICATION OF PROMOTER REGIONS

Deletion mapping of eukariotic promoter

Linker Scanning Mutagenesis Analysis

DNAse I footprinting

ChIP-Chromatin Immunoprecipitation

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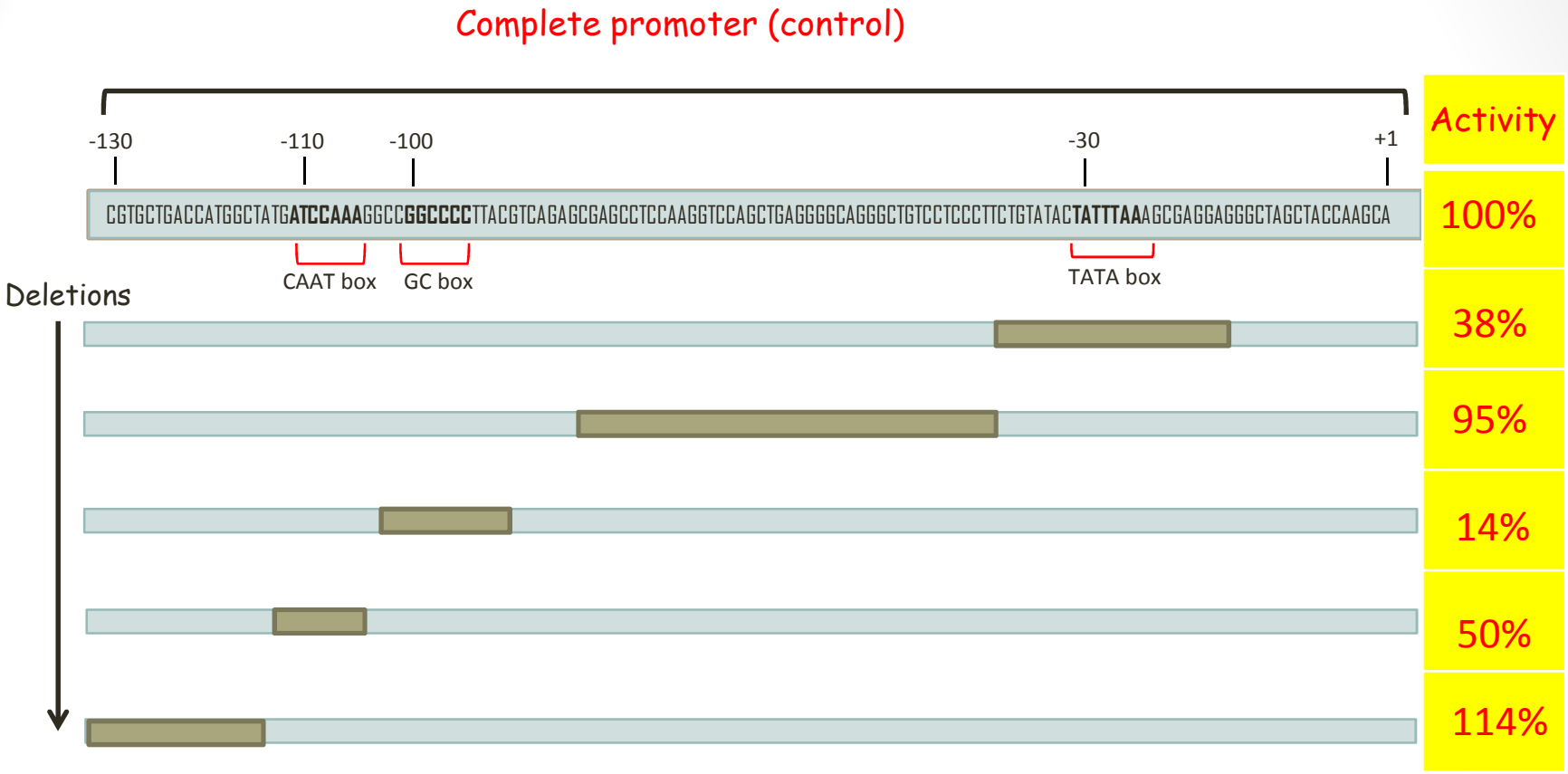

In this procedure some DNA molecules with deletions in the promoter are prepared and tested in eukariotic cells. Then it is possible to evaluate the capability of these modified promoter to stimulate gene expression.

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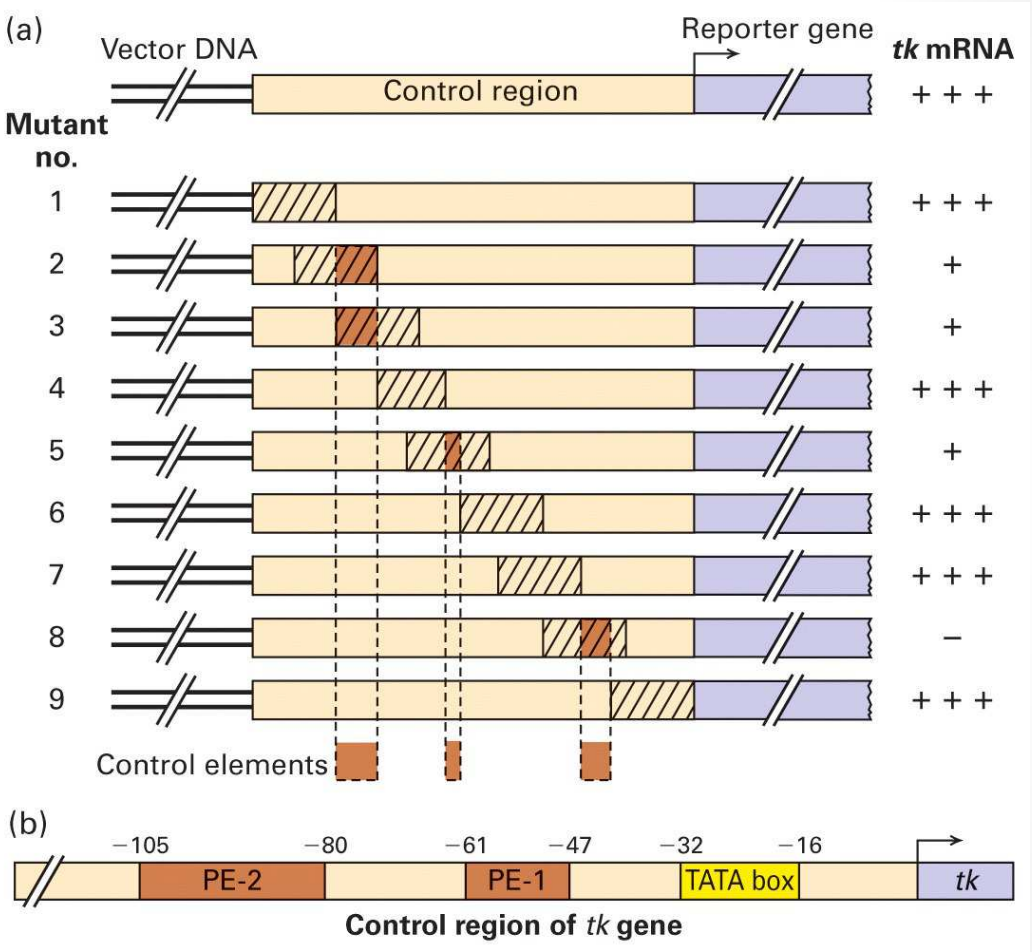
This technique is used to identify transcription control regions known as promoter-proximal elements (PE) that lie within 100-200 bp of a start site. These elements are required for transcription but are not directly involved in start site selection. PE elements are important for cell type-specific transcription of genes.



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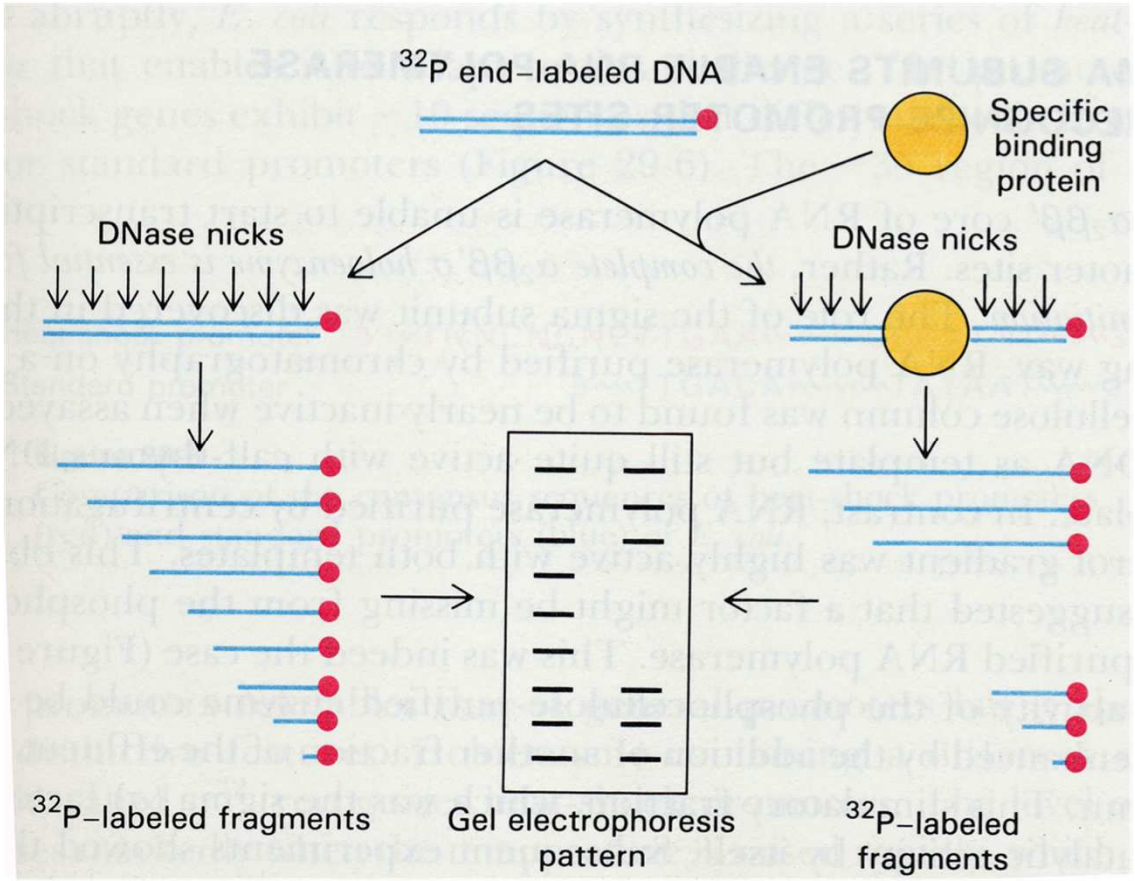

DNA labeled on one strand is incubated with the protein of interest. Then the complex is treated with a small amount of DNase I, which cleaves DNA where it is not masked by the TF. The banding patterns are compared by gel electrophoresis to locate the "footprint" region where the TF has shielded the DNA from cleavage.

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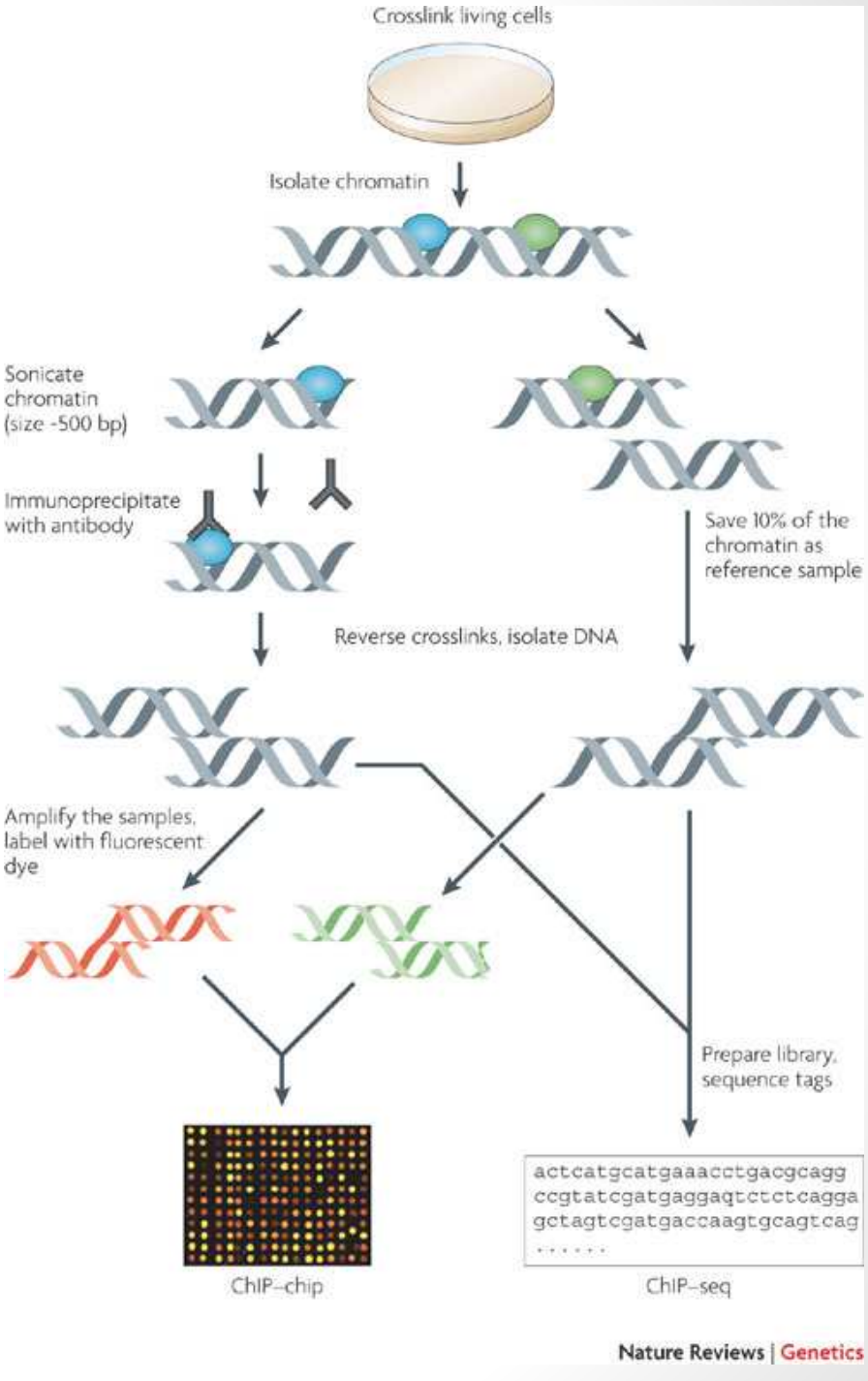
DNAse I footprinting

ChIP-Chromatin Immunoprecipitation

ChIP-Chromatin Immunoprecipitation

ChIP involves crosslinking DNA-binding proteins to DNA by treating cells with formaldehyde and preparing chromatin by sonication or enzymatic digestion. An immunoprecipitation of the crosslinked chromatin is performed using an antibody that recognizes a specific TF, which results in the identification of all the binding sites in the genome for the factor of interest. After purification of the precipitated fragments, the sample can be analysed by PCR to study particular genes.

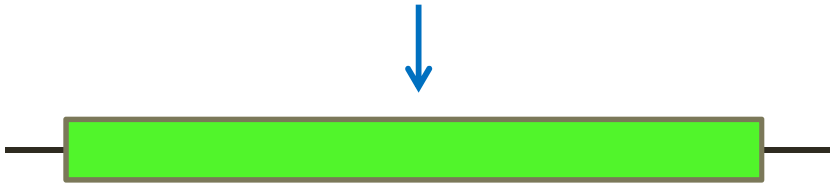


EVALUATION OF PROMOTER ACTIVITY

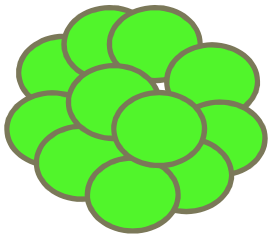
Regulatory sequence to be studied (e.g. a gene promoter)

Reporter gene (e.g.encoding GFP or luciferase)

DNA



mRNA

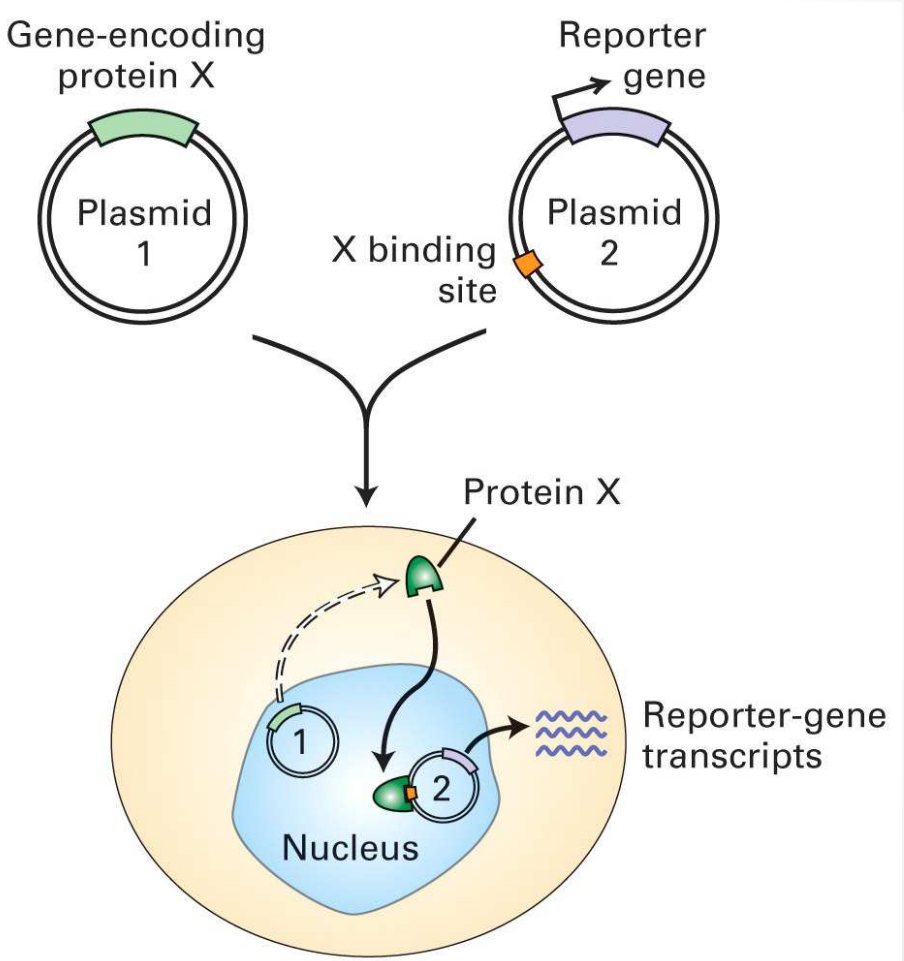


A reporter protein amount is easily measured (e.g.GFP by fluorescence)

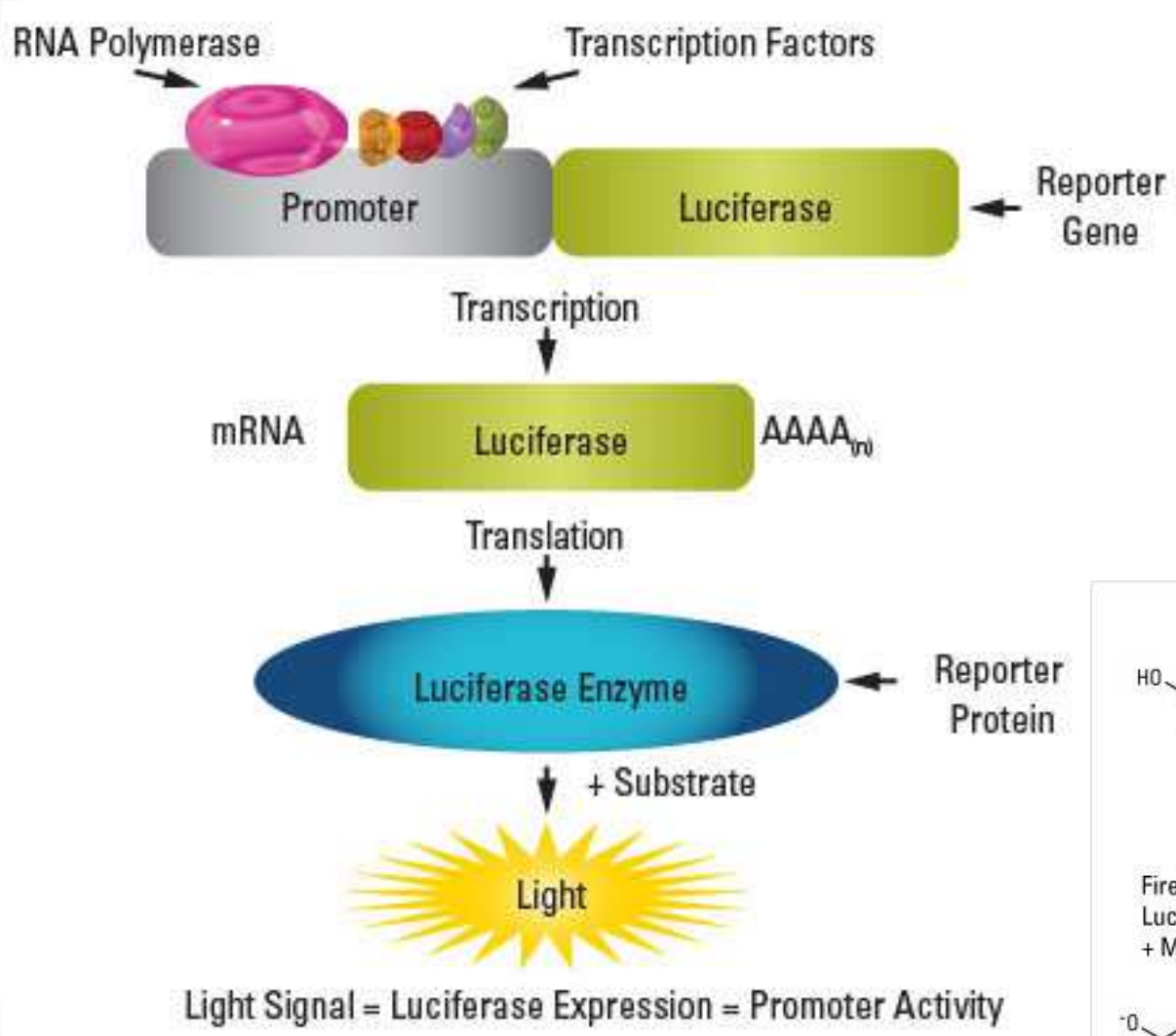
Analysis of TF activity

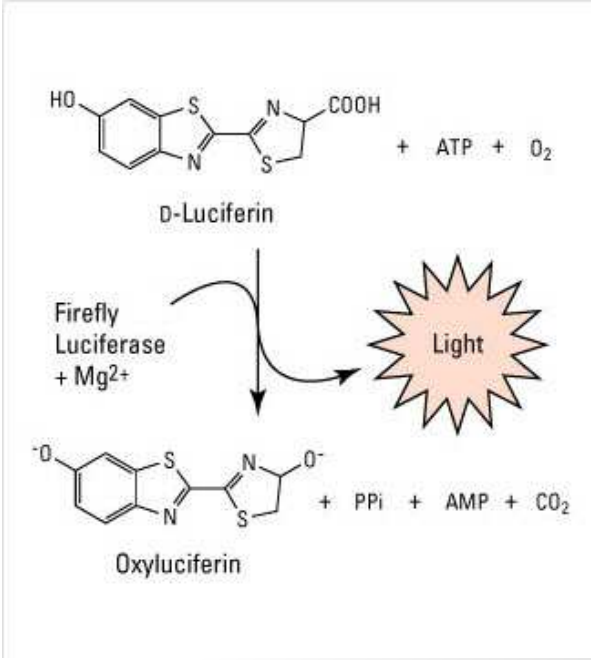
TFs can be assayed for their ability to bind to DNA control elements and regulate gene expression by transfection assays. In this method, a plasmid encoding the putative TF (protein X) is introduced into a cell along with a second vector encoding a reporter gene and the putative protein X binding site.

If protein X binds to the site and is a transcription activator, then the reporter gene is switched on. Note that the cells must not express protein X per se.

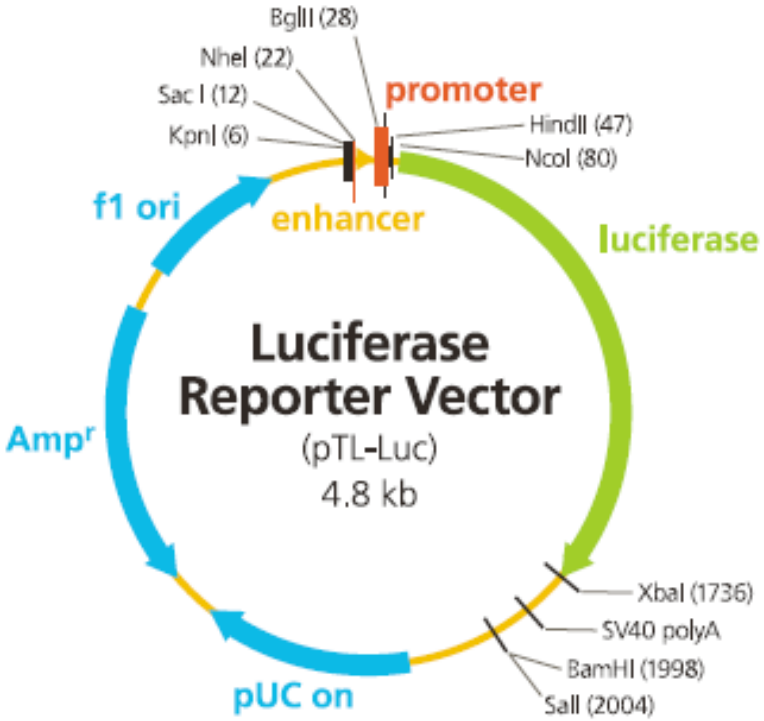


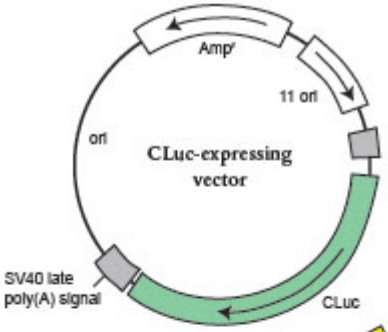
Luciferase Assay



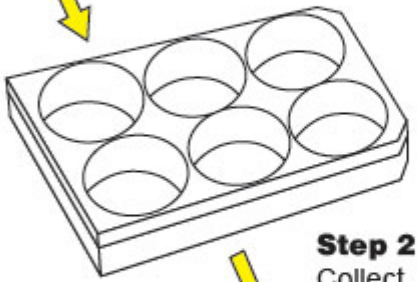


Luciferase Assay-Experimental steps

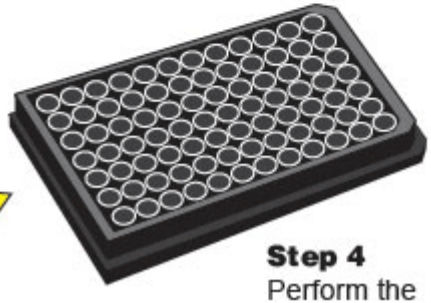




Step 1
Transfection

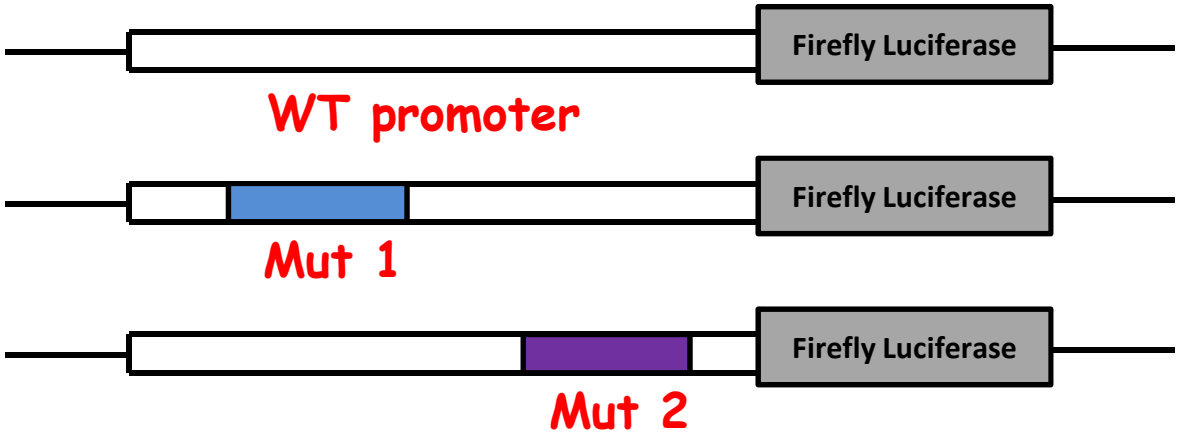


Step 2
Collect
supernatant



Step 4
Perform the
measurement

Practical example-Study of promoter mutations



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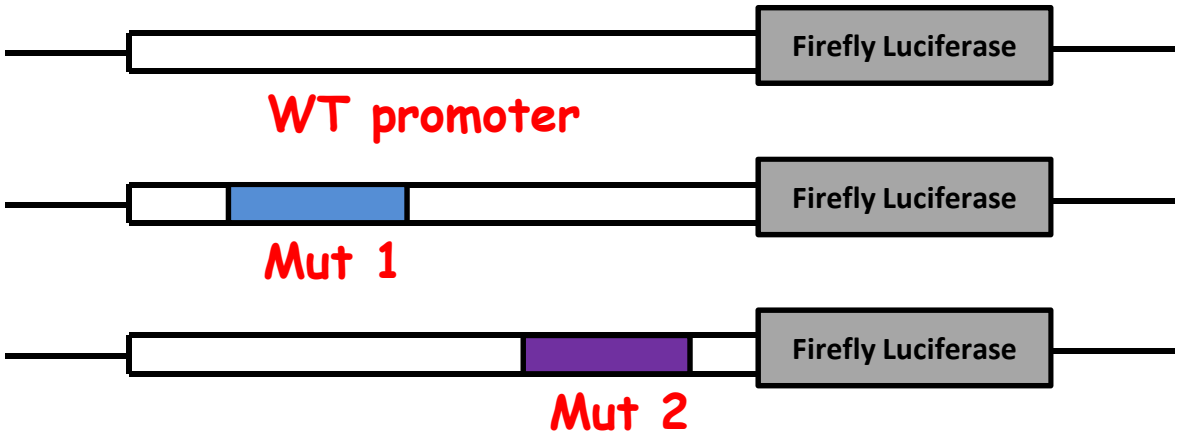
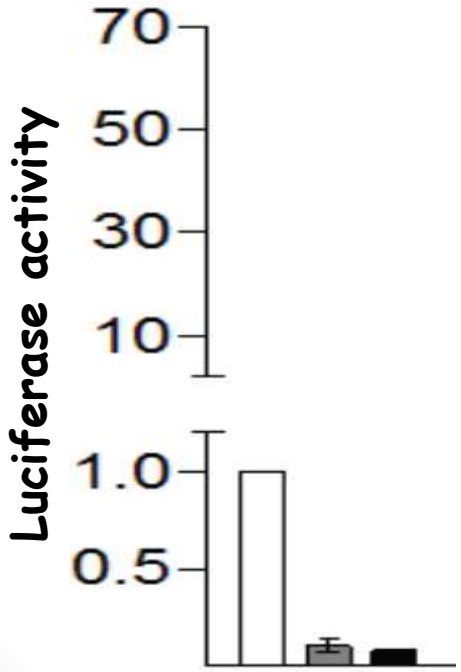




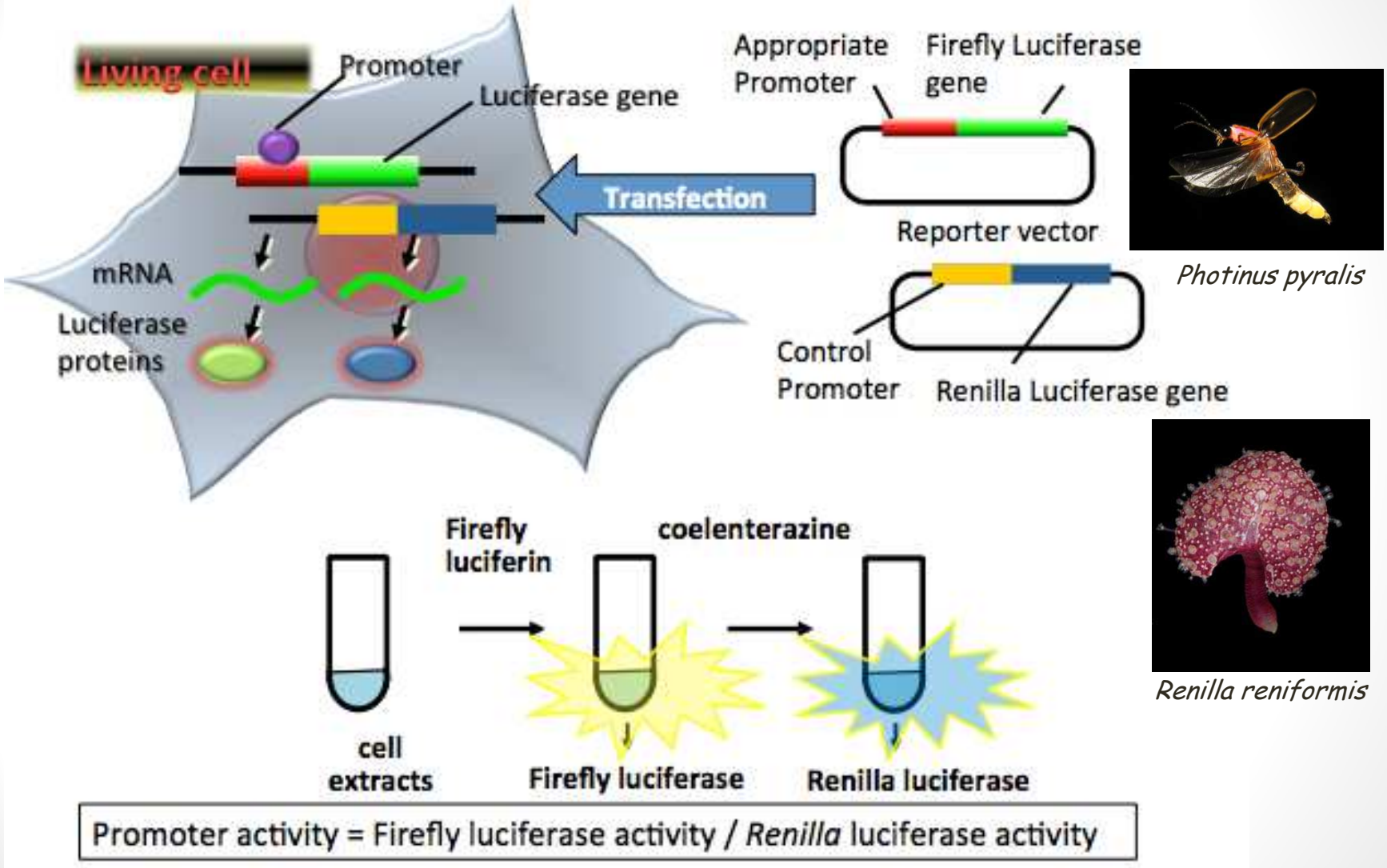
WT promoter

Mut 1

Mut 2

How can we take into account the variability due to the transfection efficiency?

Dual Luciferase Assay



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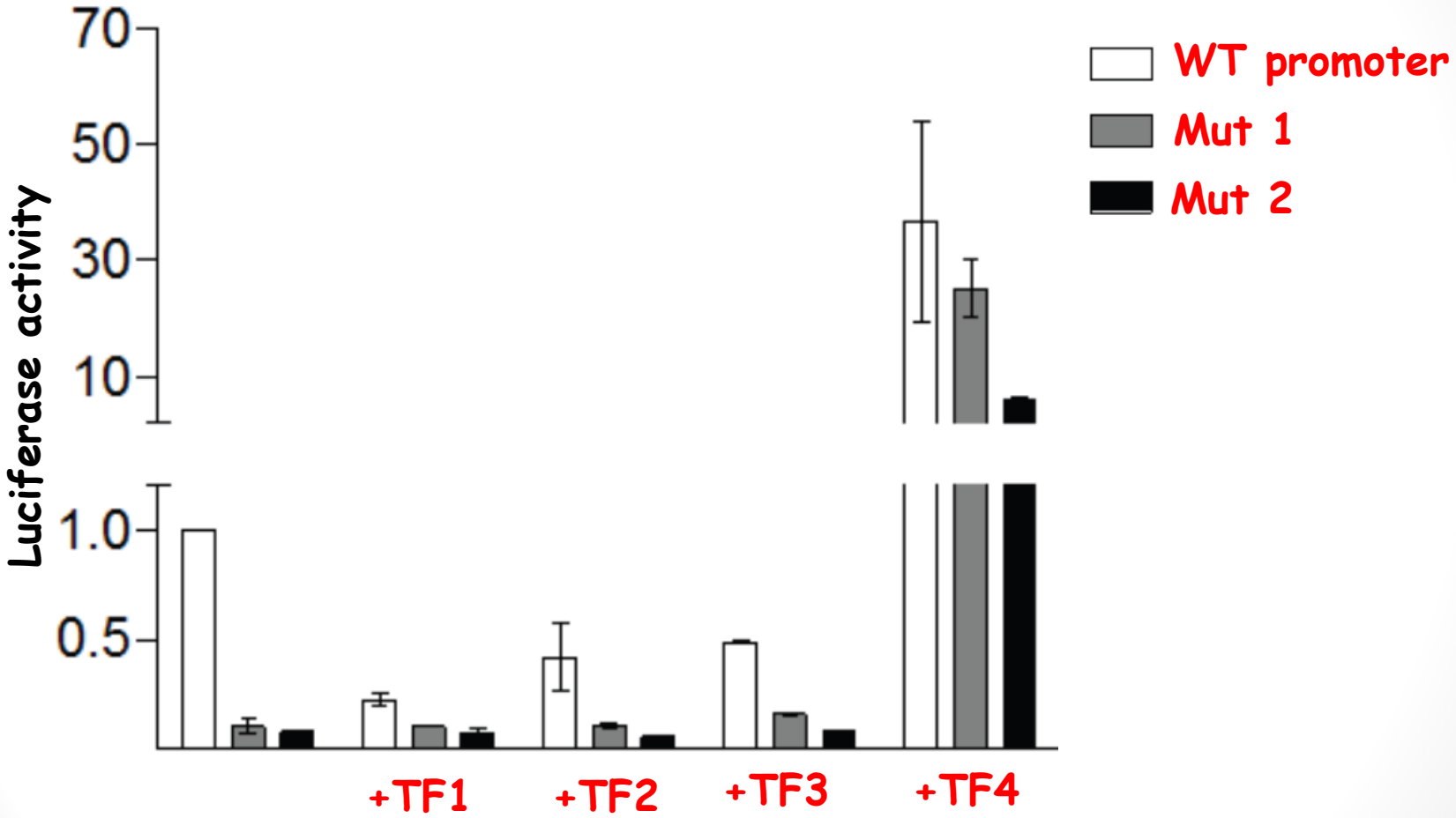

Let's interpretate the data!