Recombinant DNA-based molecular techniques (part II)

Analysis of DNA regulatory sequences and protein-protein interactions

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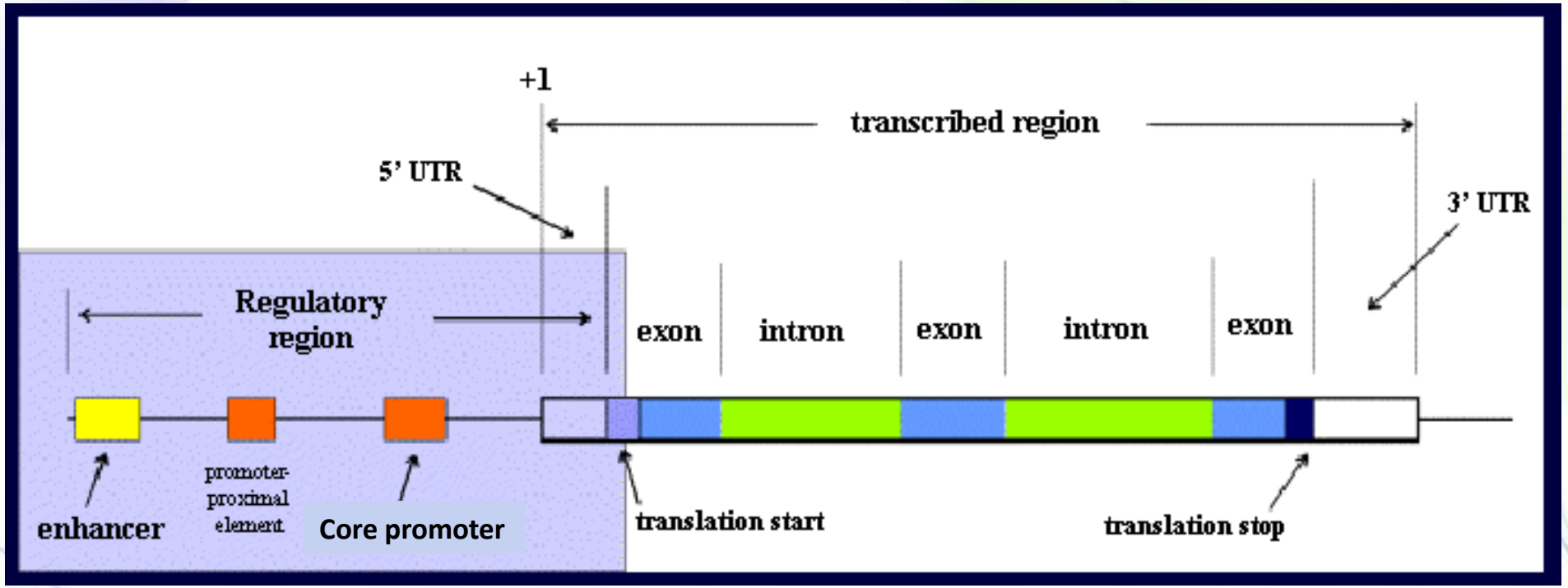
Analysis of transcriptional regulatory sequences:
Role of enzymes

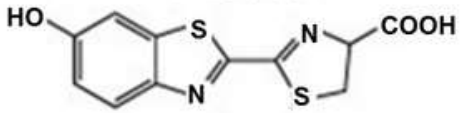
What are transcriptional regulatory sequences?



- Promoter (core promoter): A region of DNA upstream of a gene where relevant proteins (such as RNA polymerase and transcription factors) bind to initiate transcription of that gene.
- Promoter-proximal elements: Any regulatory sequence in eukaryotic DNA that is located close to (within 200 base pairs) a promoter and binds a specific protein thereby modulating transcription of the associated protein-coding gene.
- Enhancers or silencers: Regulatory DNA sequences that, when bound by specific proteins, regulate the transcription of an associated gene. They can be located near, within, after, and/or very far away from the gene, and, if lipped or relocated, are still functional.

Firefly luciferase

D-Luciferin

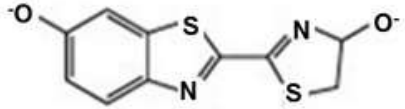


+ ATP + O_2

Firefly Luciferase + Mg^{2+}

Light

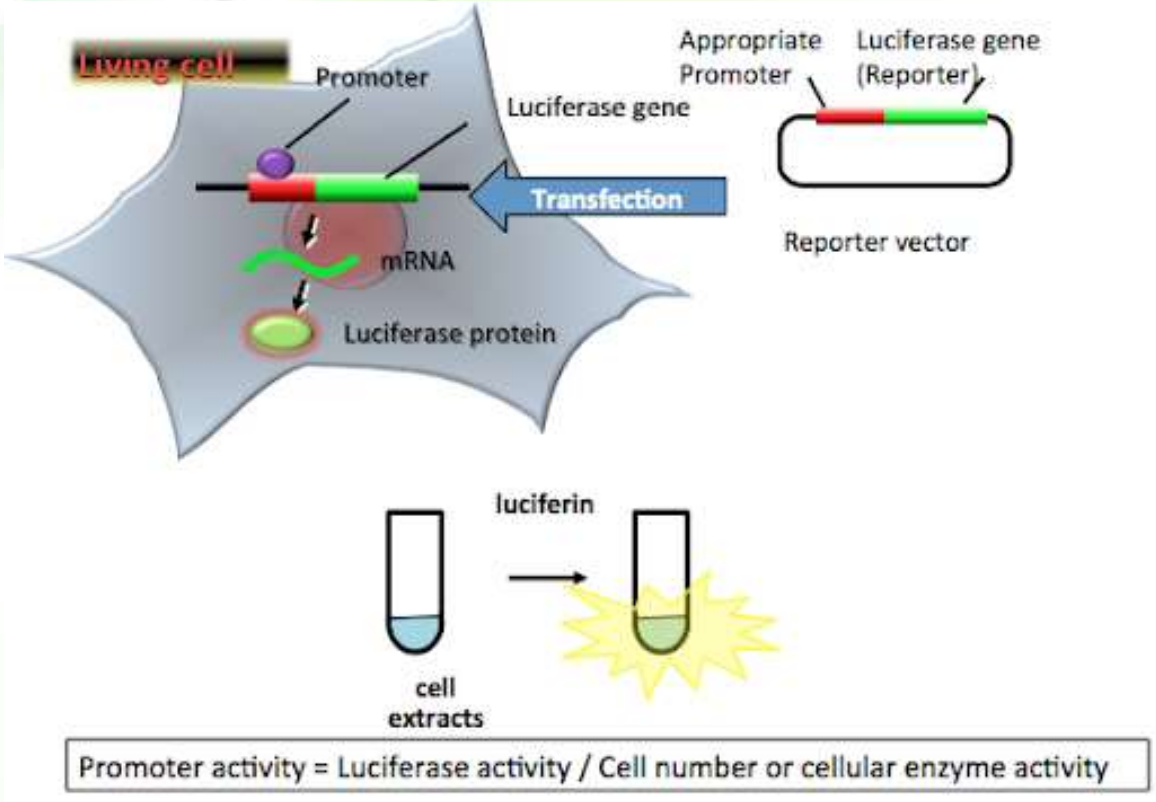
Oxyluciferin



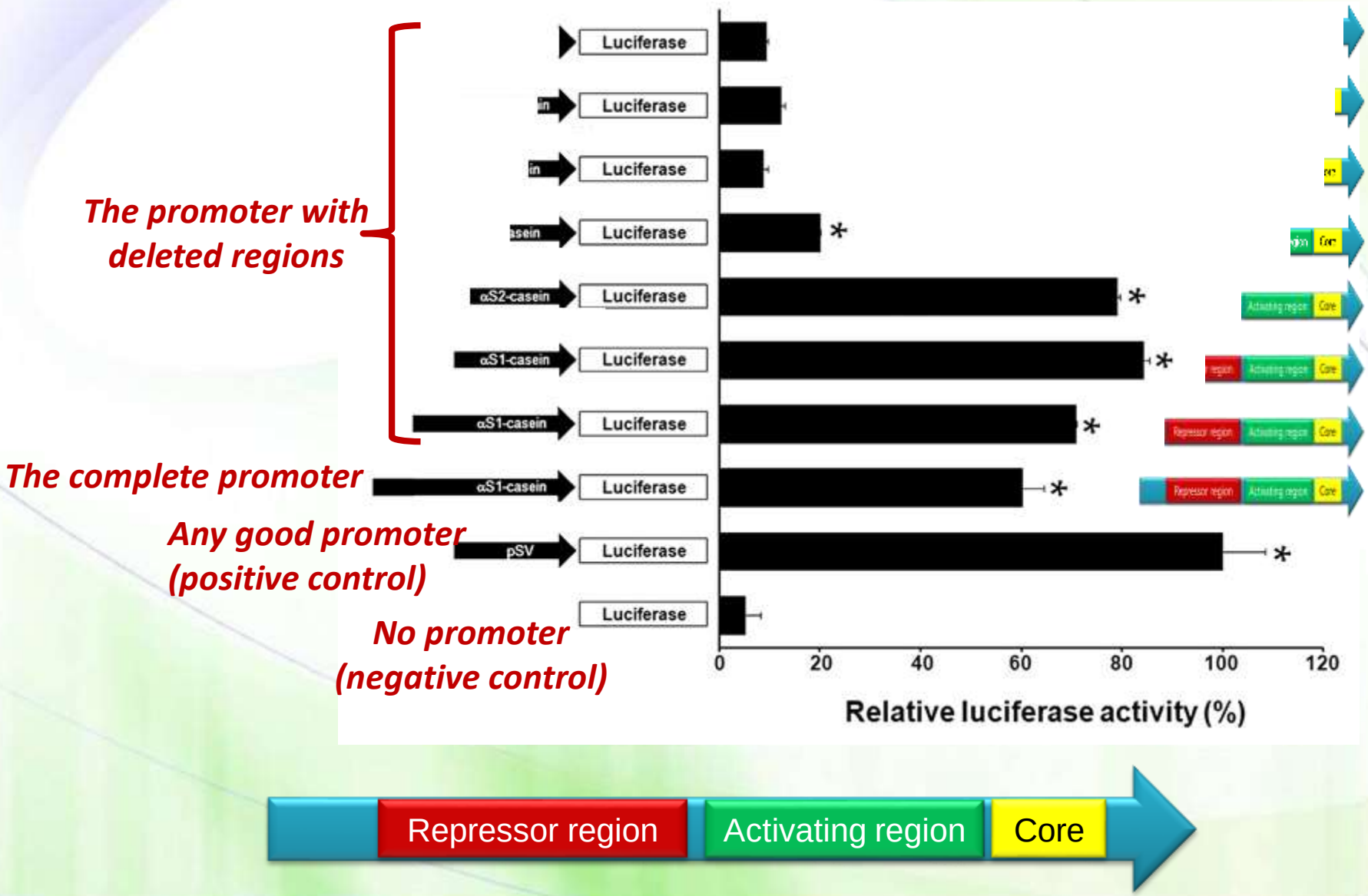
+ PPi + AMP CO_2

Luciferase reporter assay

- Purpose: study the activity of a gene at certain conditions or identify the function of certain regions of the promoter.
- **Only** the regulatory region (e.g. promoter, PPE, etc.) of the gene is placed upstream of a "reporter gene" such as the luciferase gene in a plasmid.
- The plasmid is transfected (inserted) into cells, and the expression level of luciferase (instead of the original gene itself) is measured.



Example


The promoter with deleted regions
The complete promoter
Any good promoter (positive control)
No promoter (negative control)
Luciferase
αS2-casein
αS1-casein
pSV
0
20
40
60
80
100
120
Relative luciferase activity (%)
Repressor region
Activating region
Core

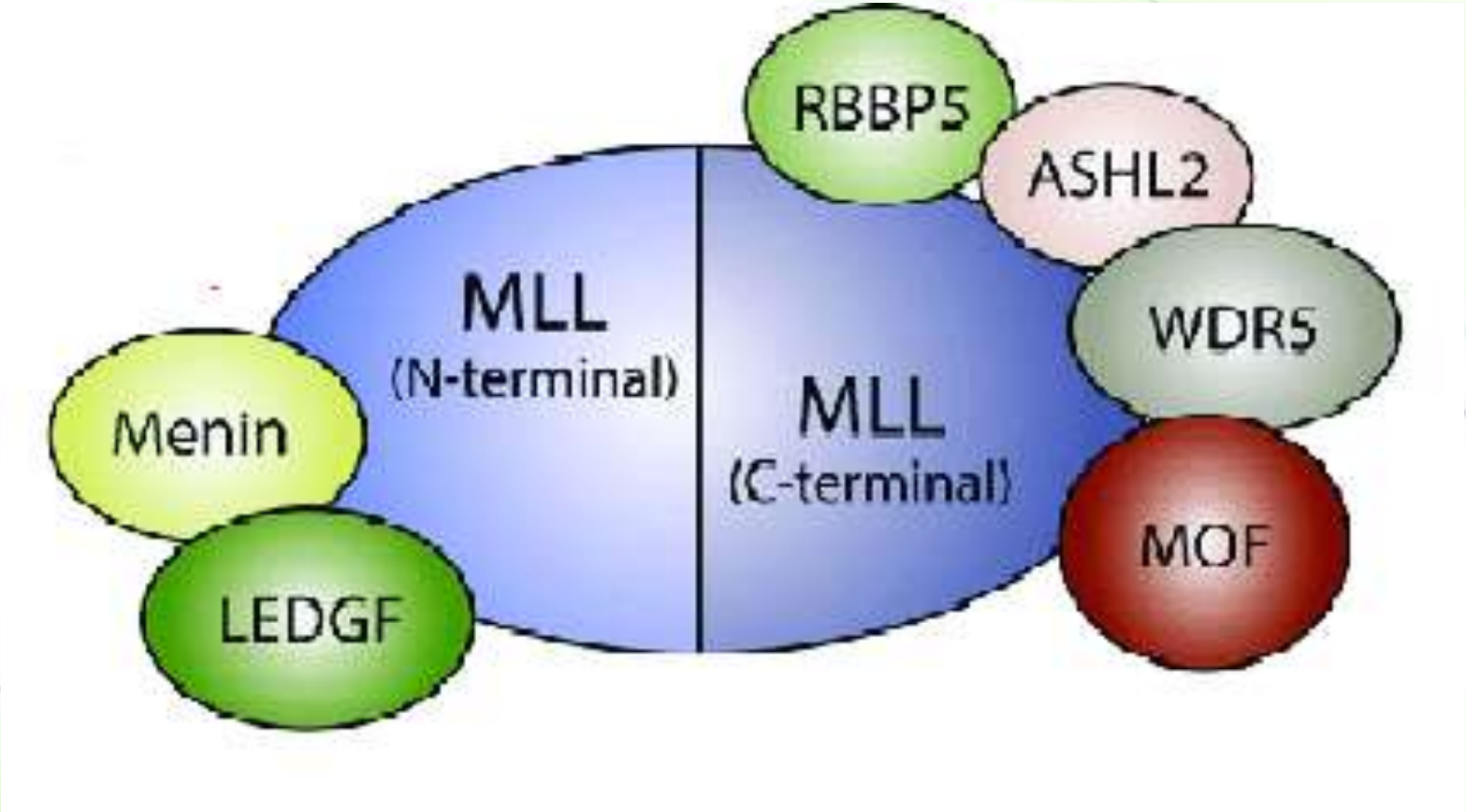
Protein-protein interaction

Co-immunoprecipitation

Yeast two-hybrid system

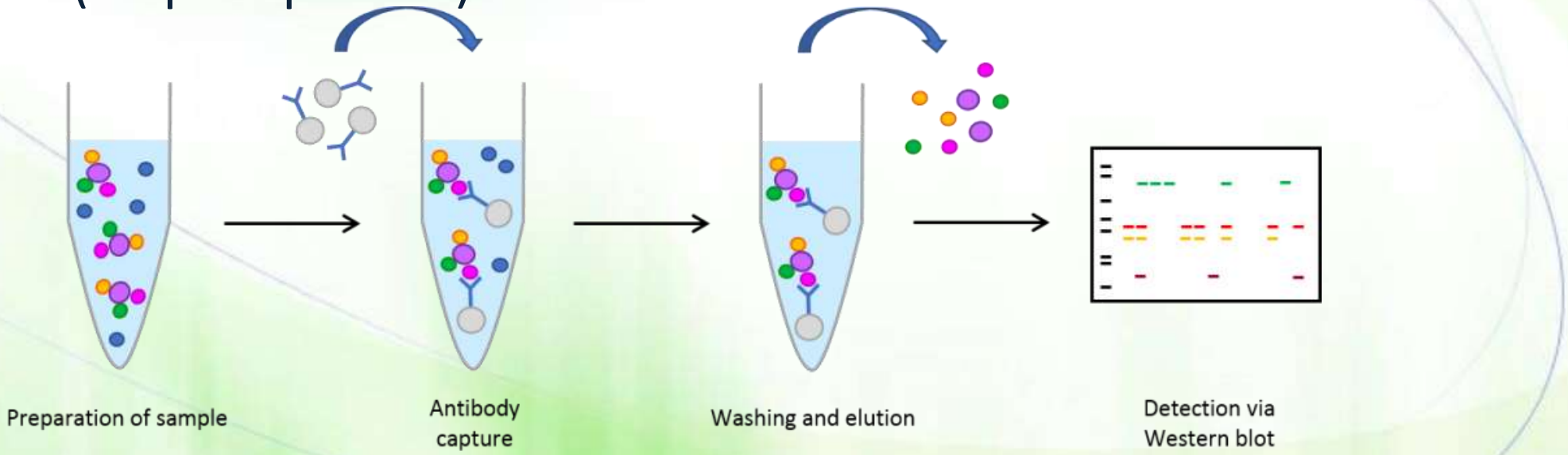
starting from a DNA library

Proteins form complexes



(Co)-Immunoprecipitation

- Antibody molecules that target a specific protein are conjugated to special beads.
- A mixture of cell proteins are added to the beads.
- Only the protein of interest is precipitated as well as other proteins bound to it (co-precipitated).

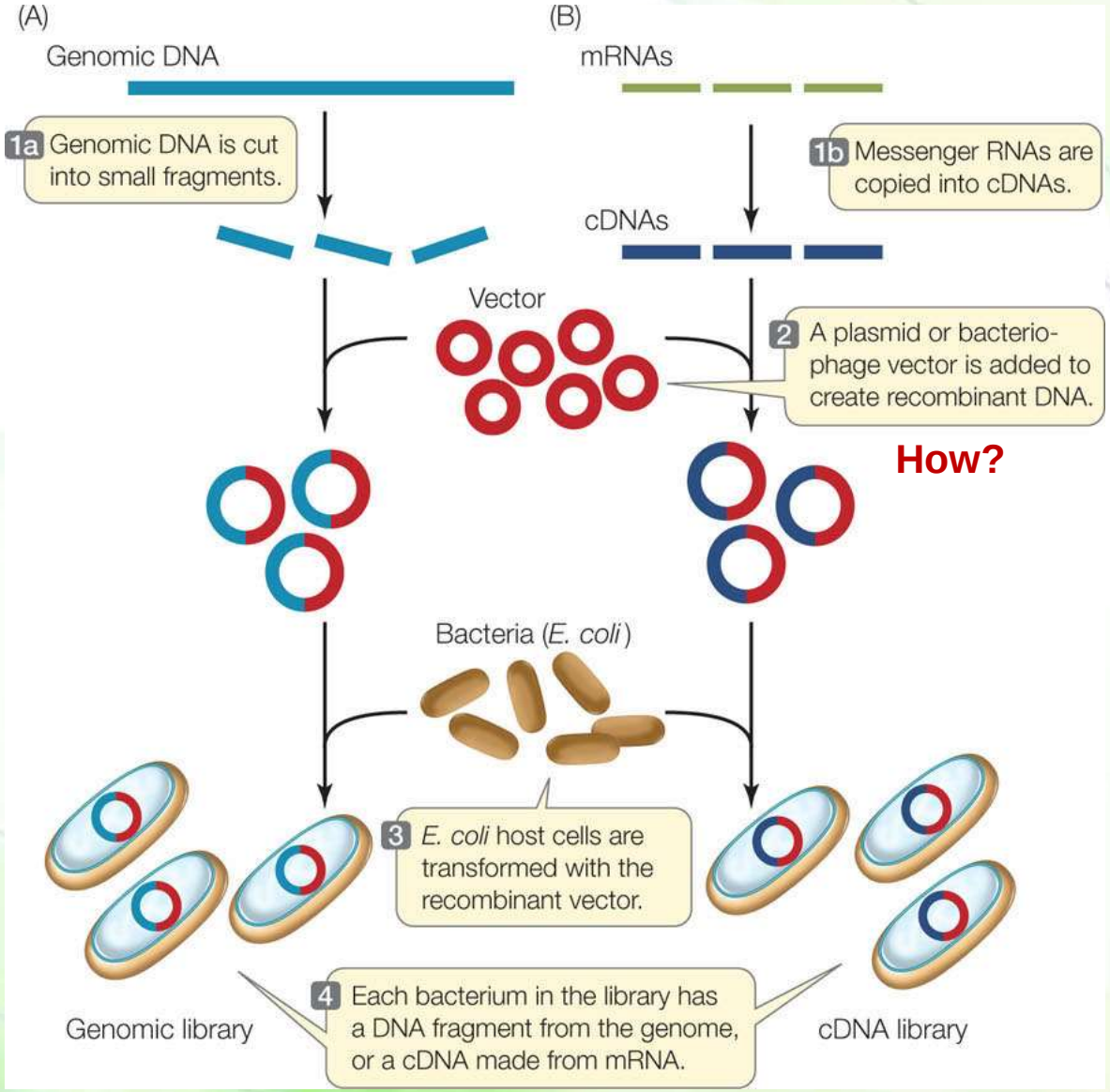


What is a DNA library?

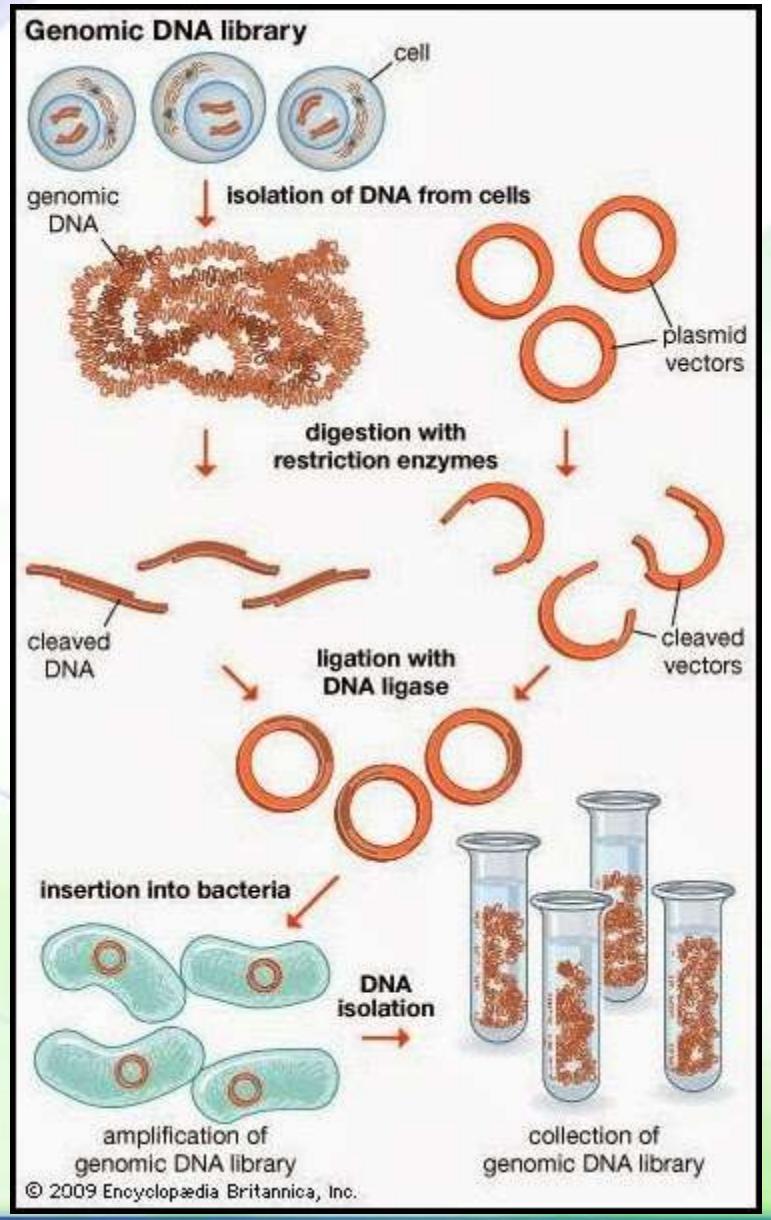
- A library can be created for DNA fragments just like book libraries.
- You can have clones of bacteria each containing a specific piece of DNA.
- You can save these clones in the freezer and take whichever clone you want to study.

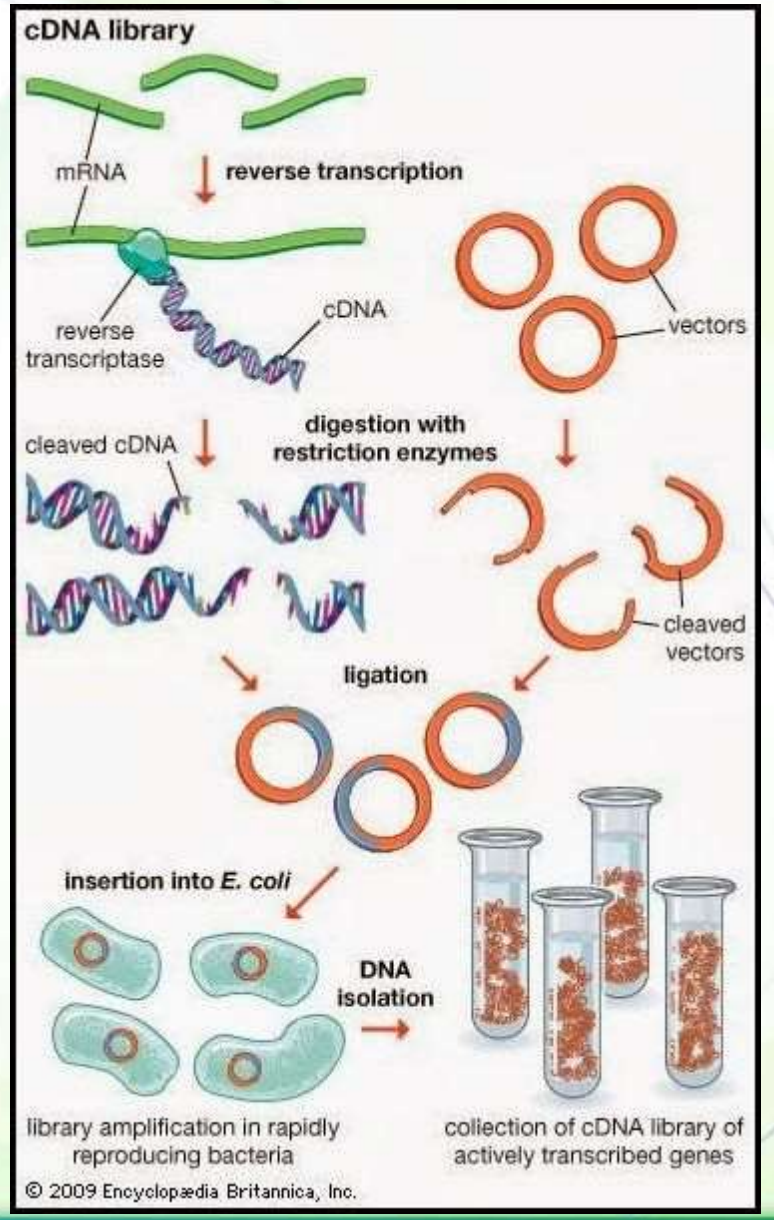
- http://www.sumanasinc.com/webcontent/animations/content/dnalibrary.html

Genomic vs. cDNA libraries



Genomic vs. cDNA libraries

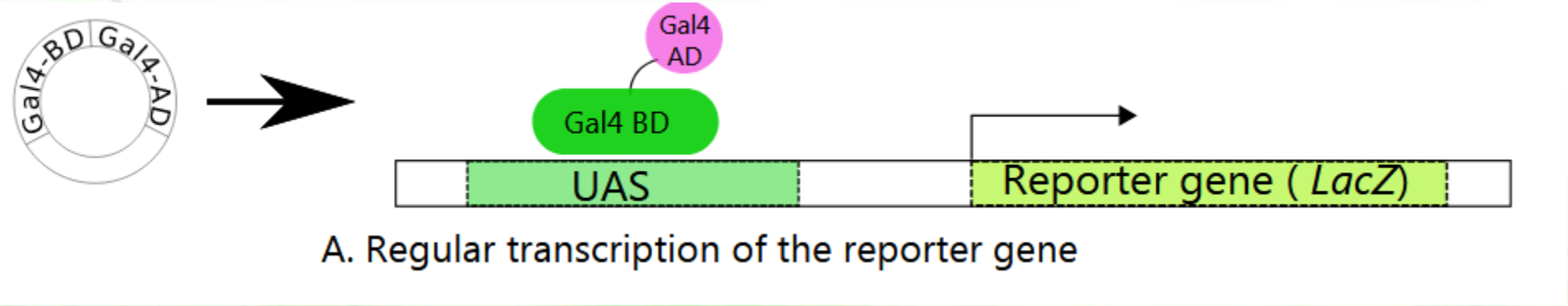




Yeast two-hybrid system

Taking advantage of protein domains

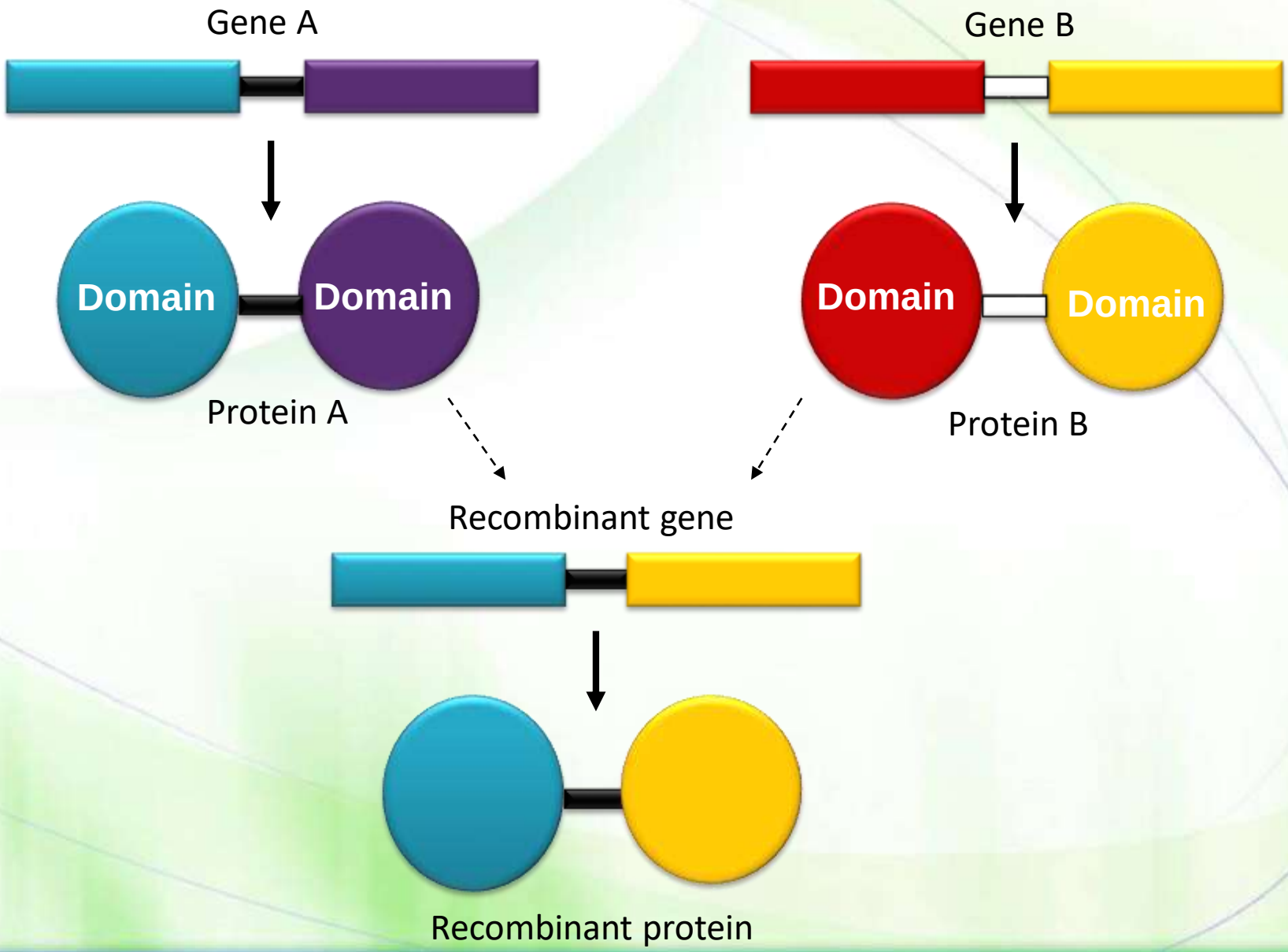
- In yeast, an upstream activating sequence (UAS) exists.
- UAS is controlled by a transcription factor that is made of two domains
  - A DNA-binding domain (BD)
  - An activation domain (AD) that is responsible for the activation of transcription.
  - Both must be close to each other in order to transcribe a reporter gene such the LacZ gene.



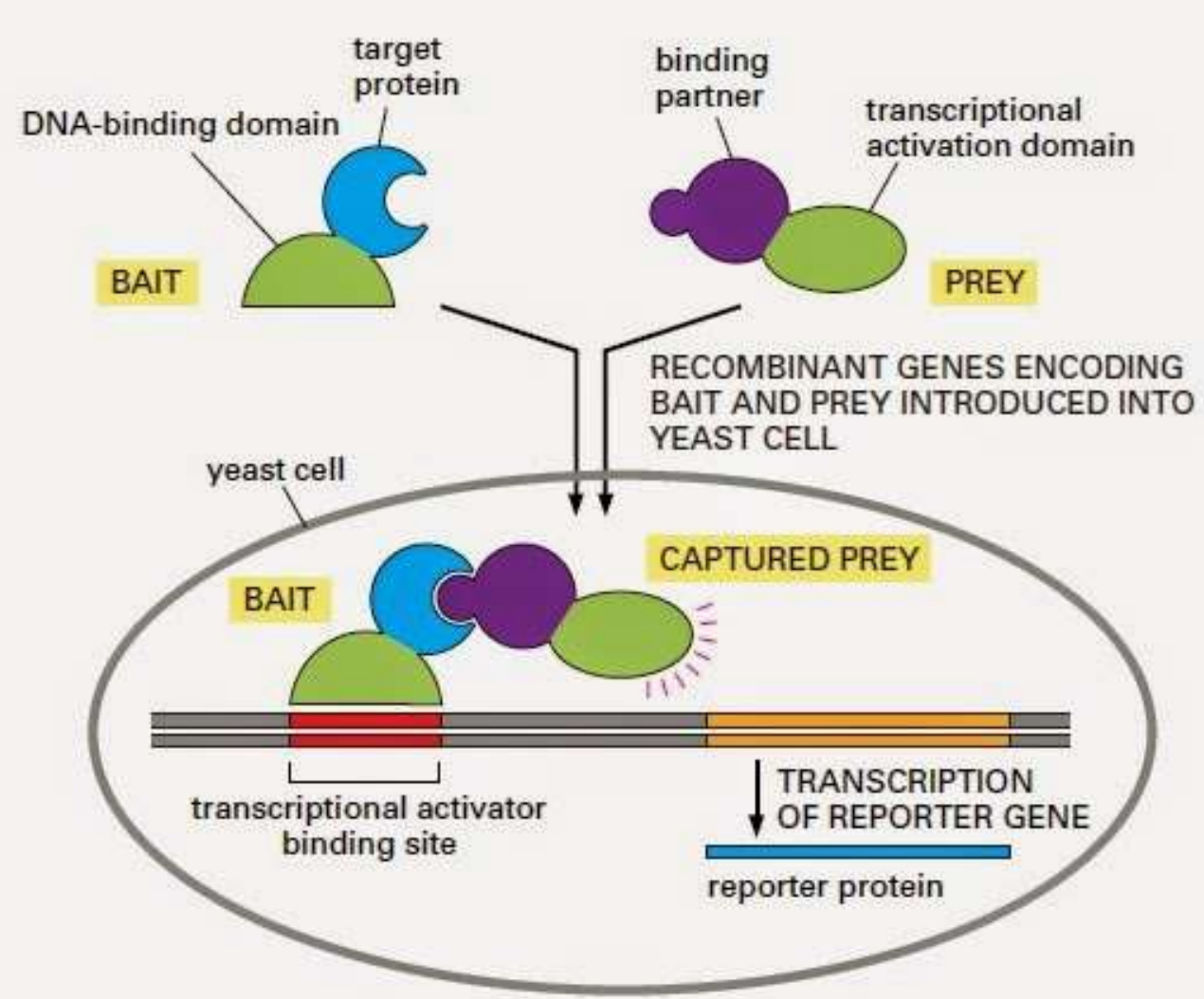
A. Regular transcription of the reporter gene

https://www.youtube.com/watch?v=okxle_hTaZ0
https://www.youtube.com/watch?v=NxNfibcNk_Y

Production of a recombinant protein



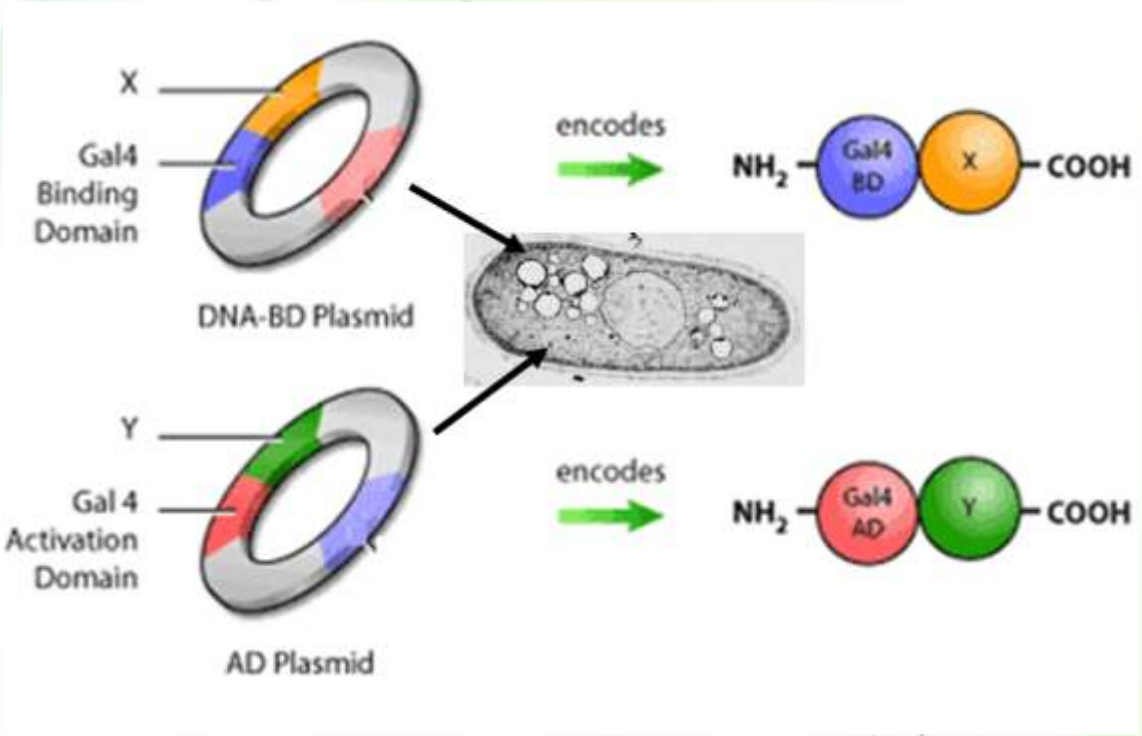
Quick illustration



Yeast Two Hybrid System

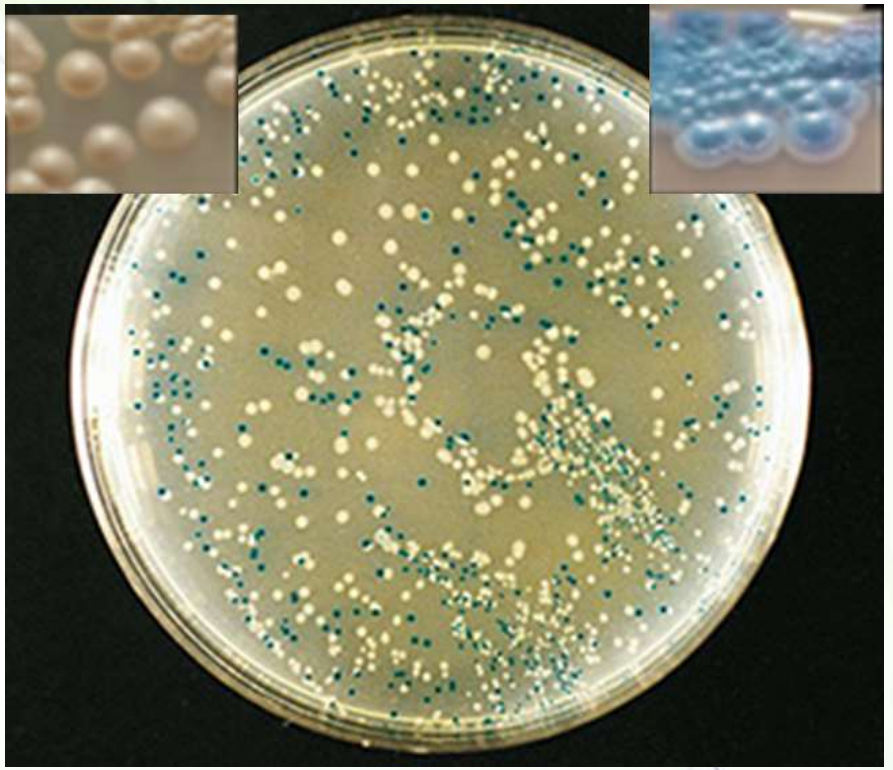
Cloning of hybrid proteins

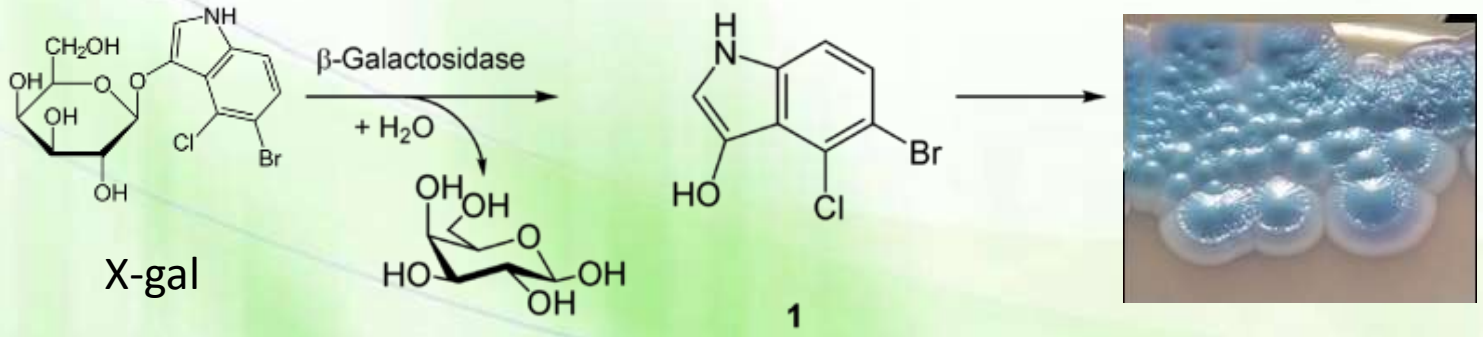
- In order to discover unknown proteins (Y's) that interact with a known protein (X), the X gene is cloned so it is produced recombined with the DB domain and the unknown Y gene (or genes) are separately cloned so that they are produced recombined with AD.
- Both recombinant plasmids are transferred into yeast cells so <u>all</u> of them express the known X gene-BD hybrid, but <u>each one</u> expresses a different unknown Y gene-AD hybrid.



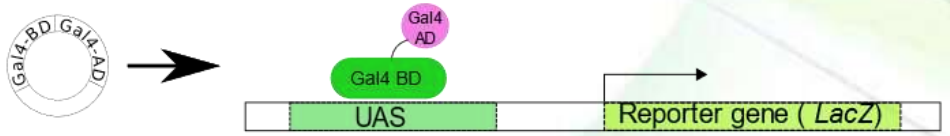
Why is the LacZ gene used? What is X-gal?

- Yeast cells are grown in the presence of a lactose analog called X-gal, which generates a blue product when cleaved.
- When the LacZ gene is activated, beta-galactosidase is produced, which cleaves X-gal generating blue colonies.



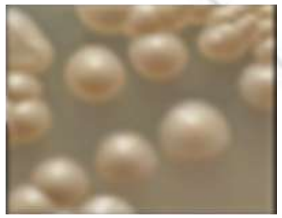


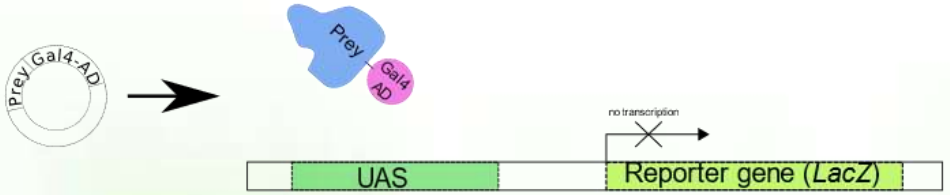
The possibilities and outcomes



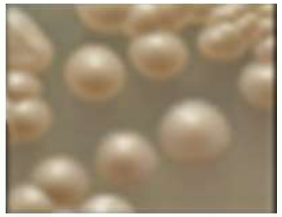
A. Regular transcription of the reporter gene

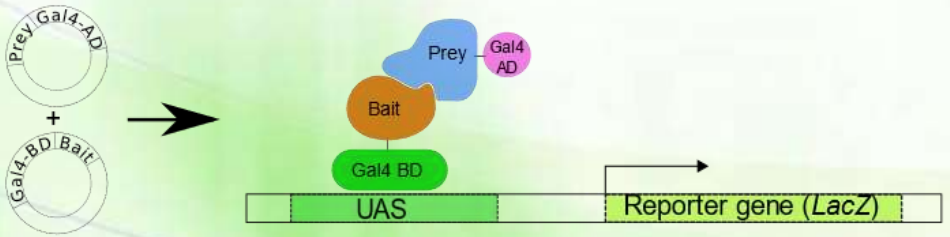
B. One fusion protein only (Gal4-BD + Bait) - no transcription



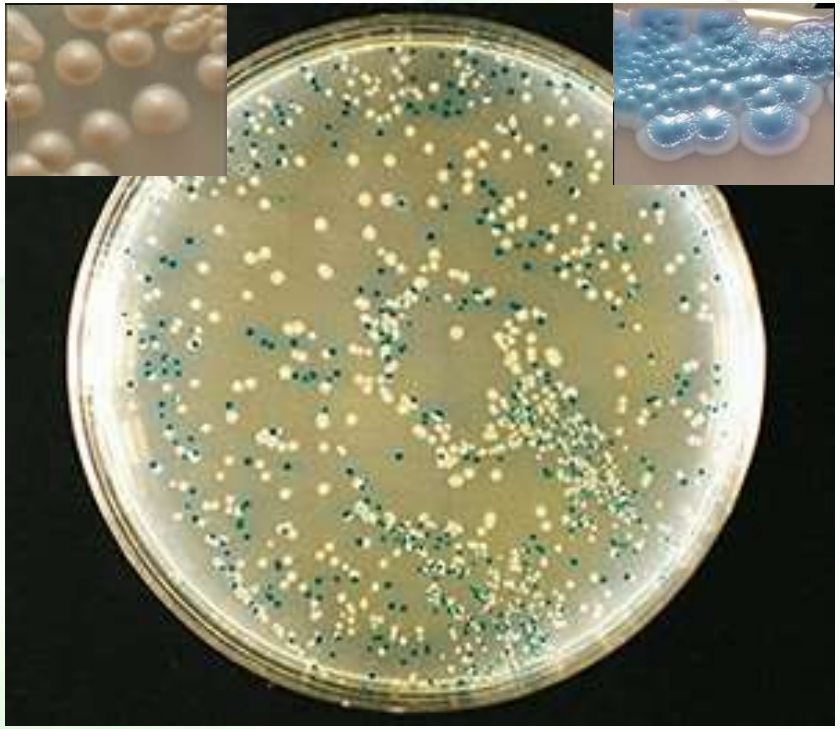


C. One fusion protein only (Gal4-AD + Prey) - no transcription





D. Two fusion proteins with interacting Bait and Prey



- Blue yeast colonies are picked and plasmids are isolated to identify the unknown genes/proteins that interact with the known gene/protein.