Molecular Biology (12)
Recombinant proteins

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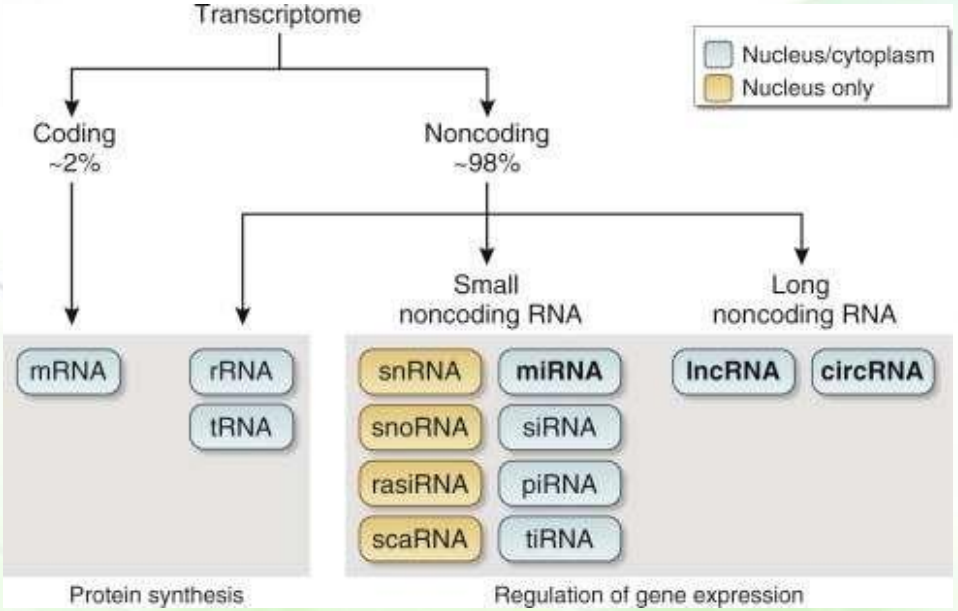
Expression of human proteins in bacteria

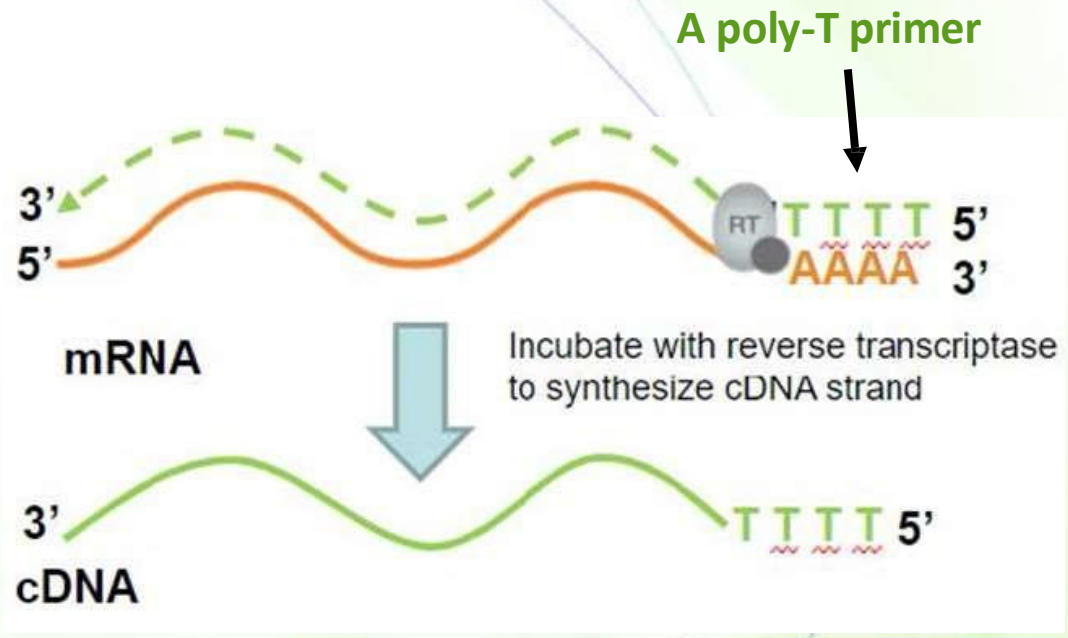
How do we select for human mRNA?

The power of reverse transcriptase (part 1)

The "many types of RNA" challenge



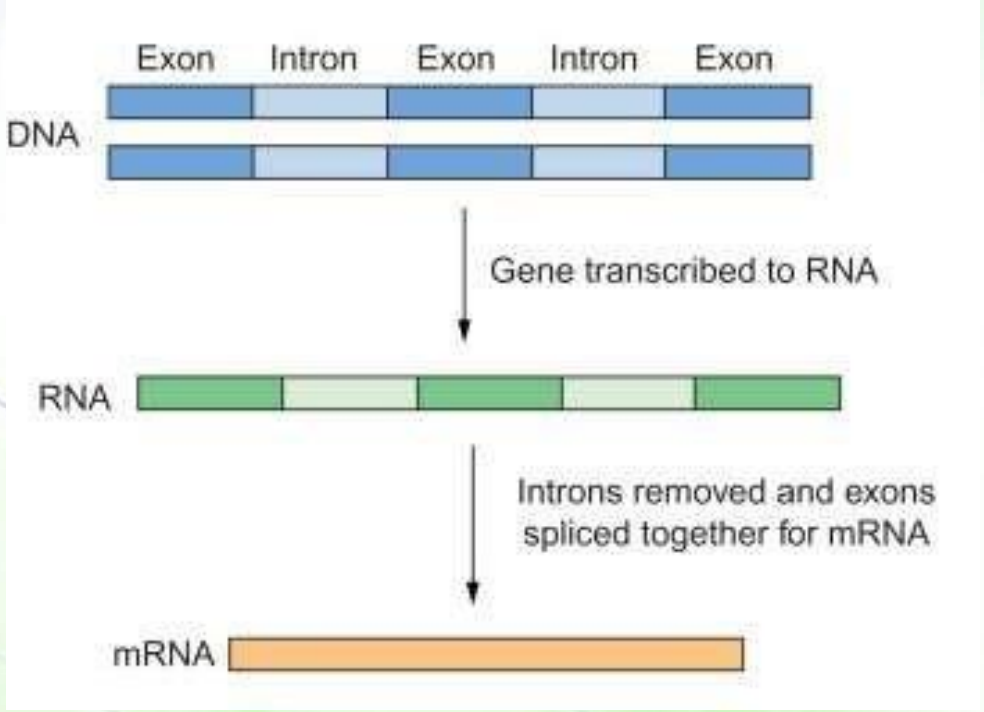
The "poly-T primer" solution



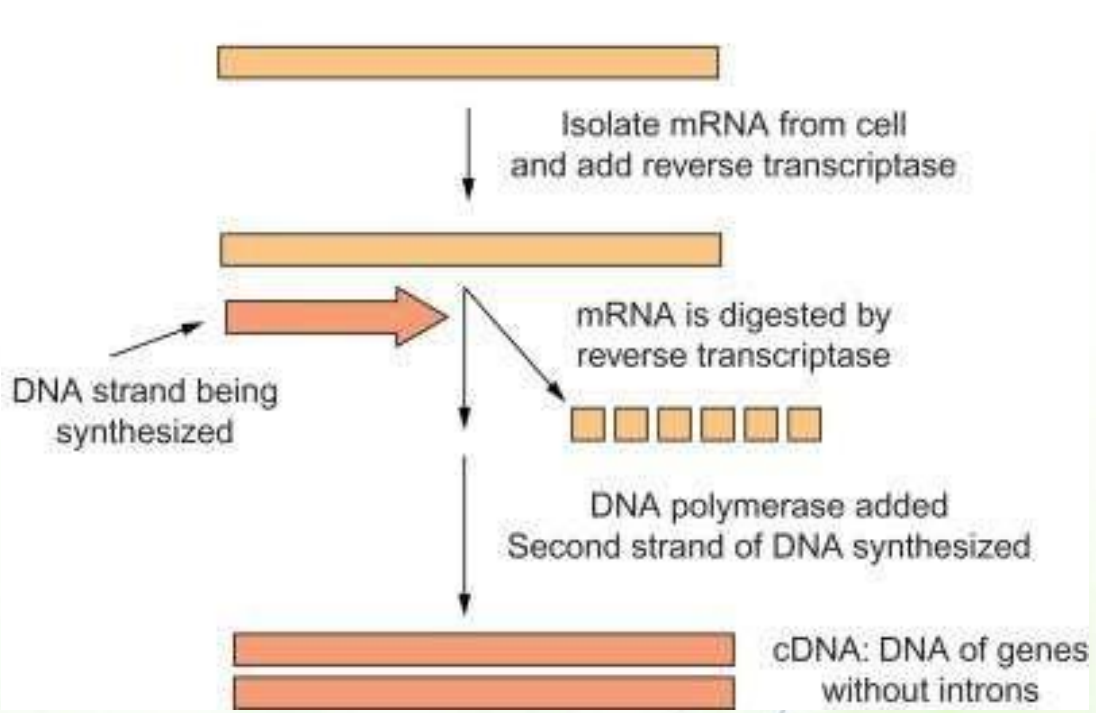
How do we deselect introns?

The power of reverse transcriptase (part 2)

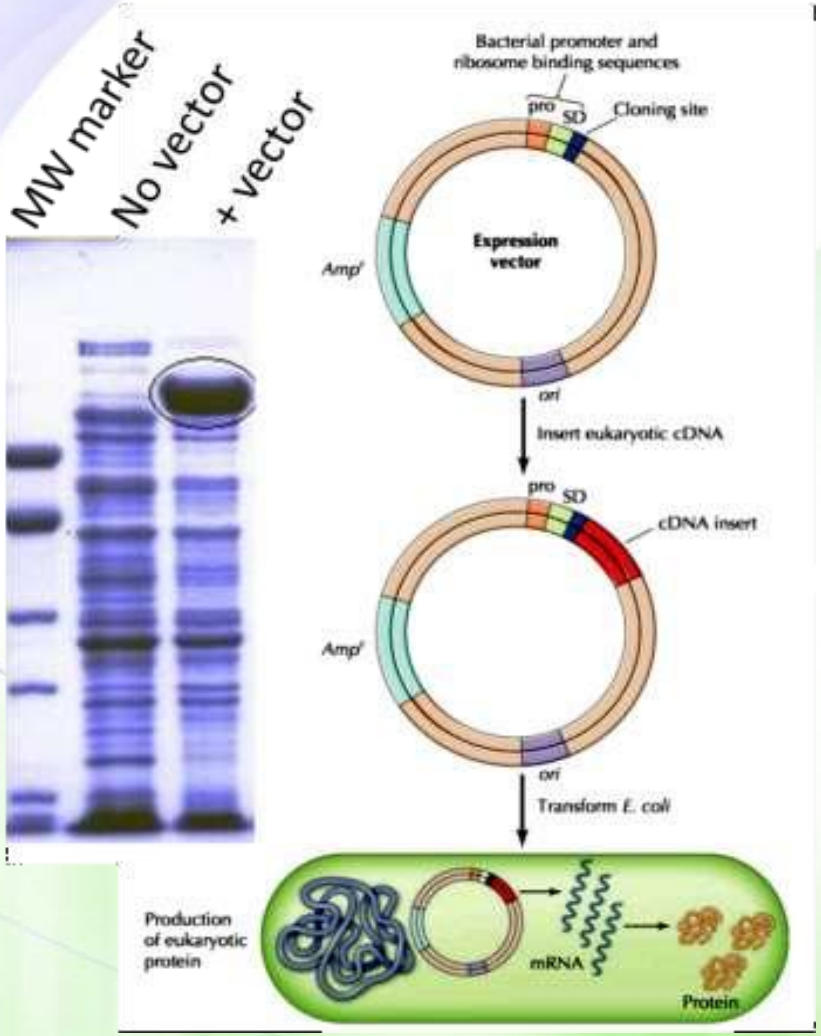
Go for mature mRNA



The "reverse" solution



Expression vectors

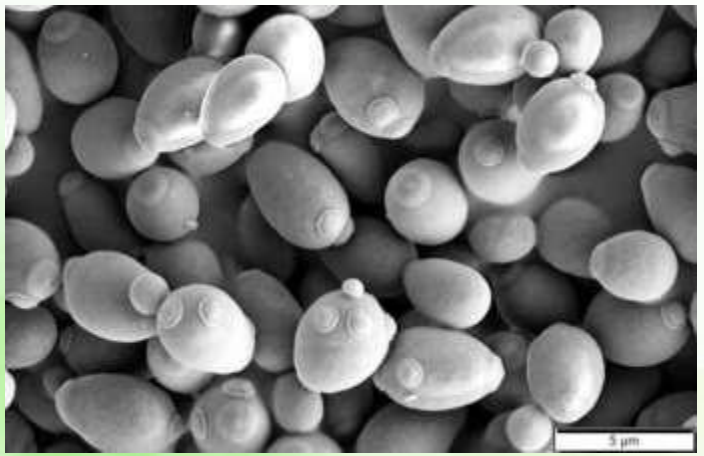


- Expression vectors contain additional sequences:
  - Promoter sequences upstream of gene to be inserted,
  - Ribosomal binding sequences (Shine-Dalgarno [SD] sequences),
  - A transcription termination sequence.
- The protein is expressed and purified.
- Examples: insulin, growth hormone, plasminogen activator, erythropoietin

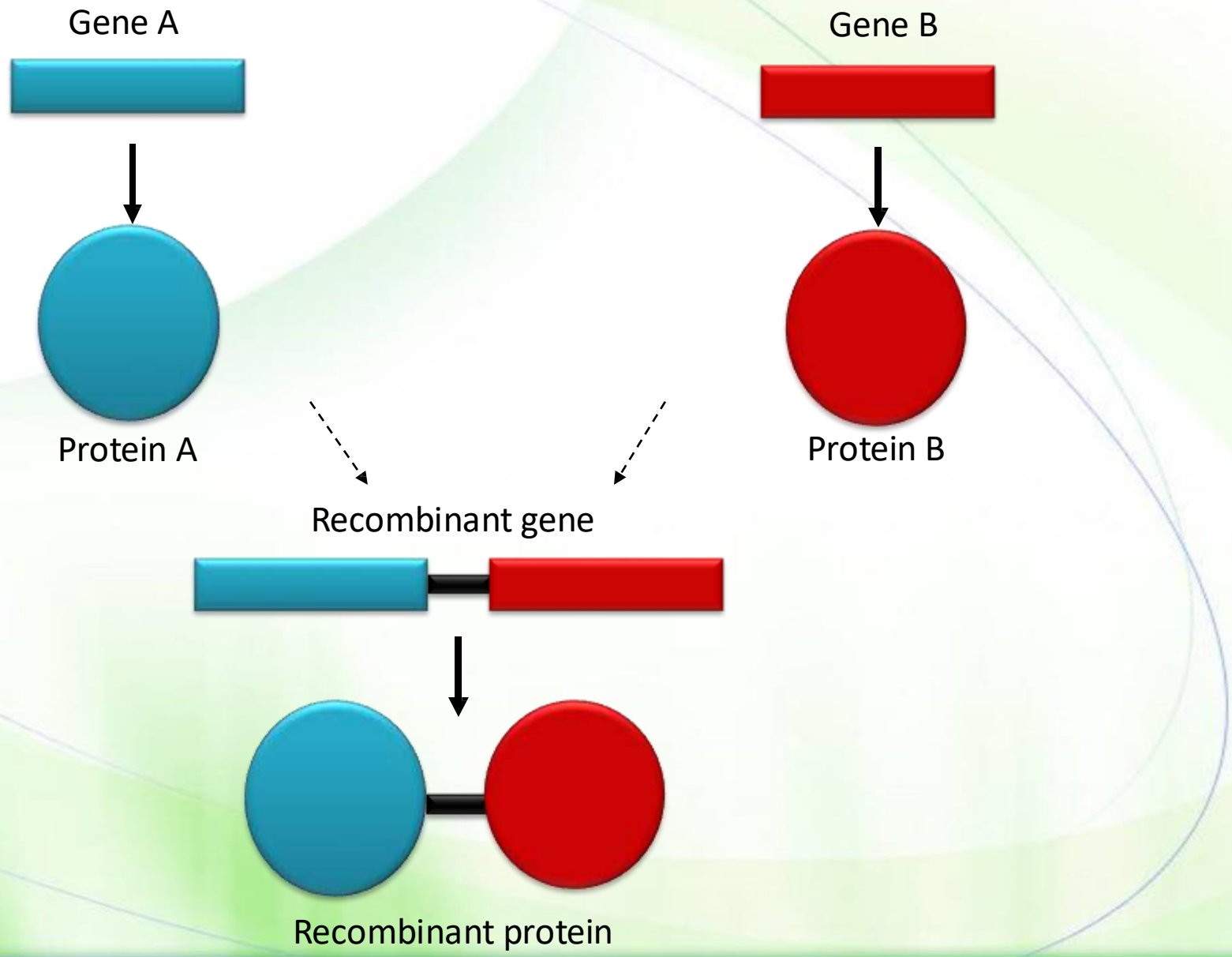
Challenges of protein expression in bacteria

- No internal disulfide bonds
- No post-translational modification (example: glycosylation)
- Protein misfolding
- Protein degradation

- Solution: use a eukaryotic system such as yeast

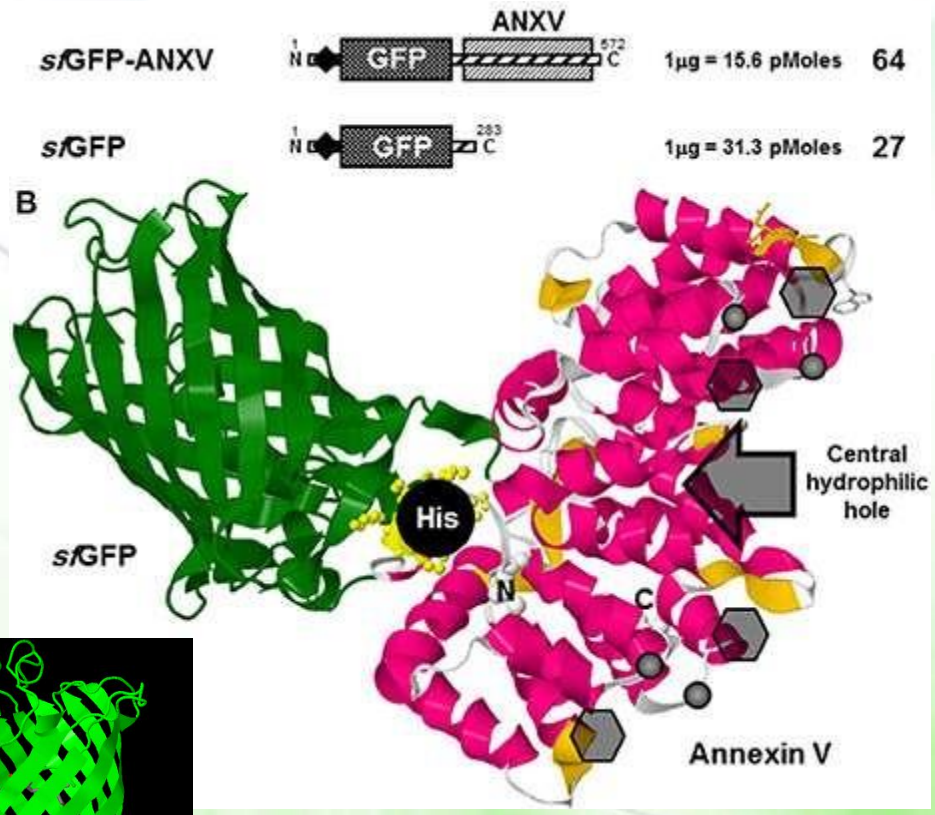


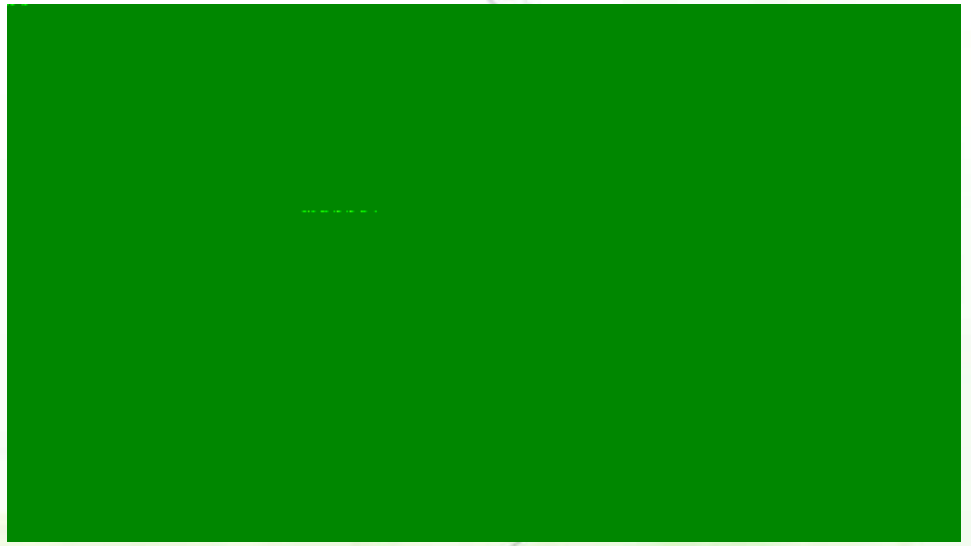
Production of a recombinant protein

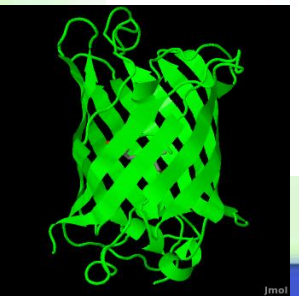


GFP-tagged proteins

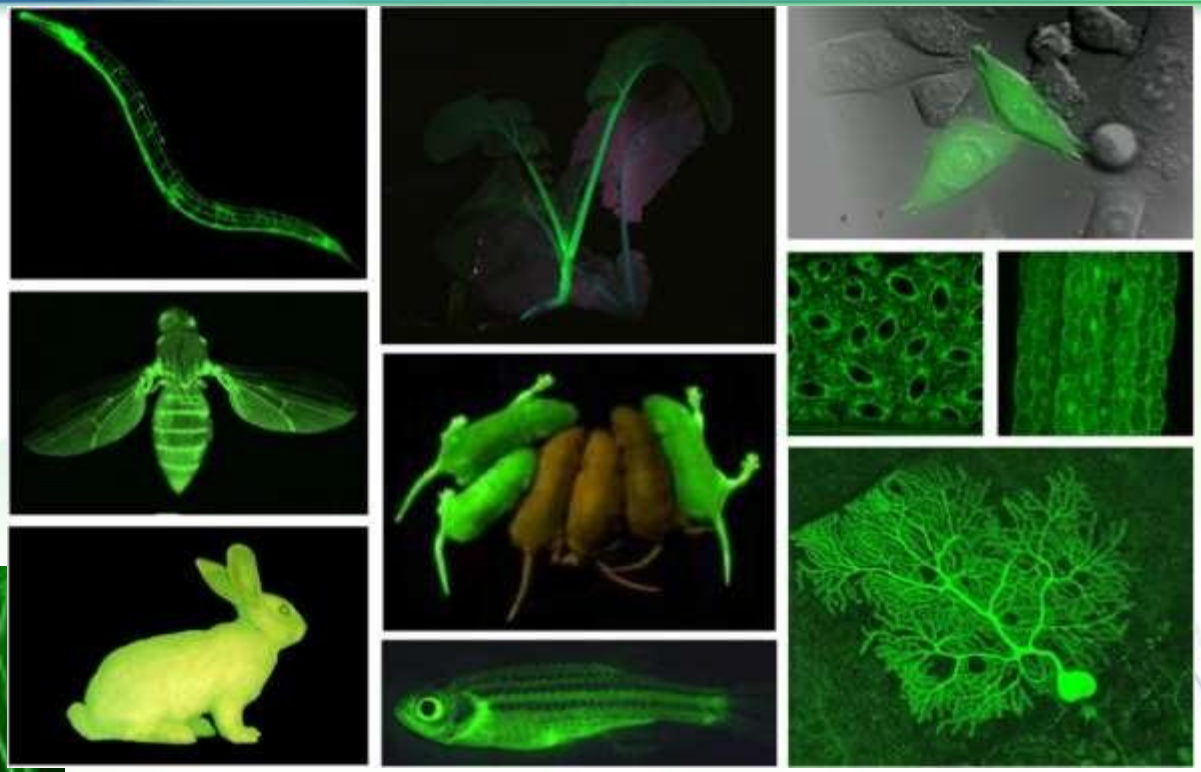
- Green Fluorescent Protein (GFP) allows for protein detection rather than for purification purposes.

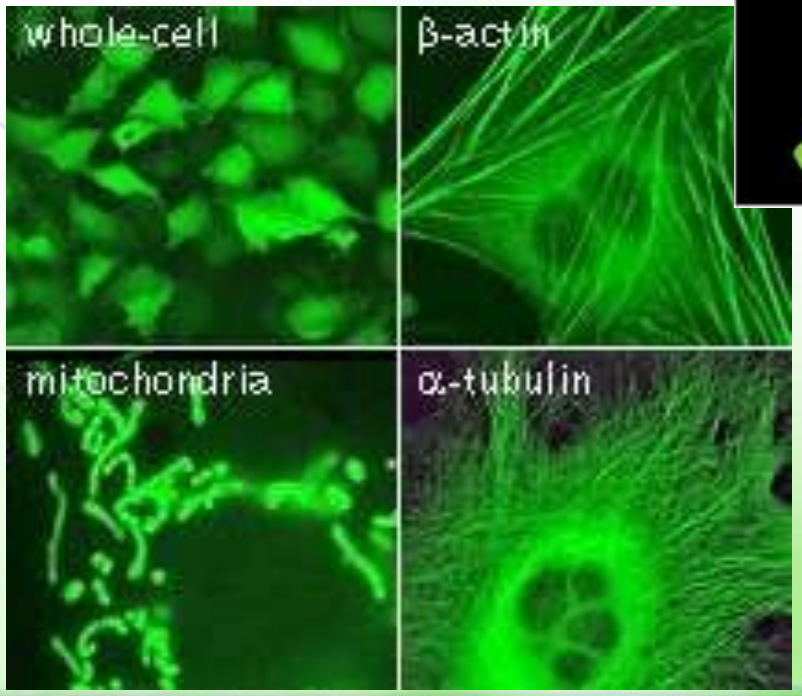






A world of possibilities

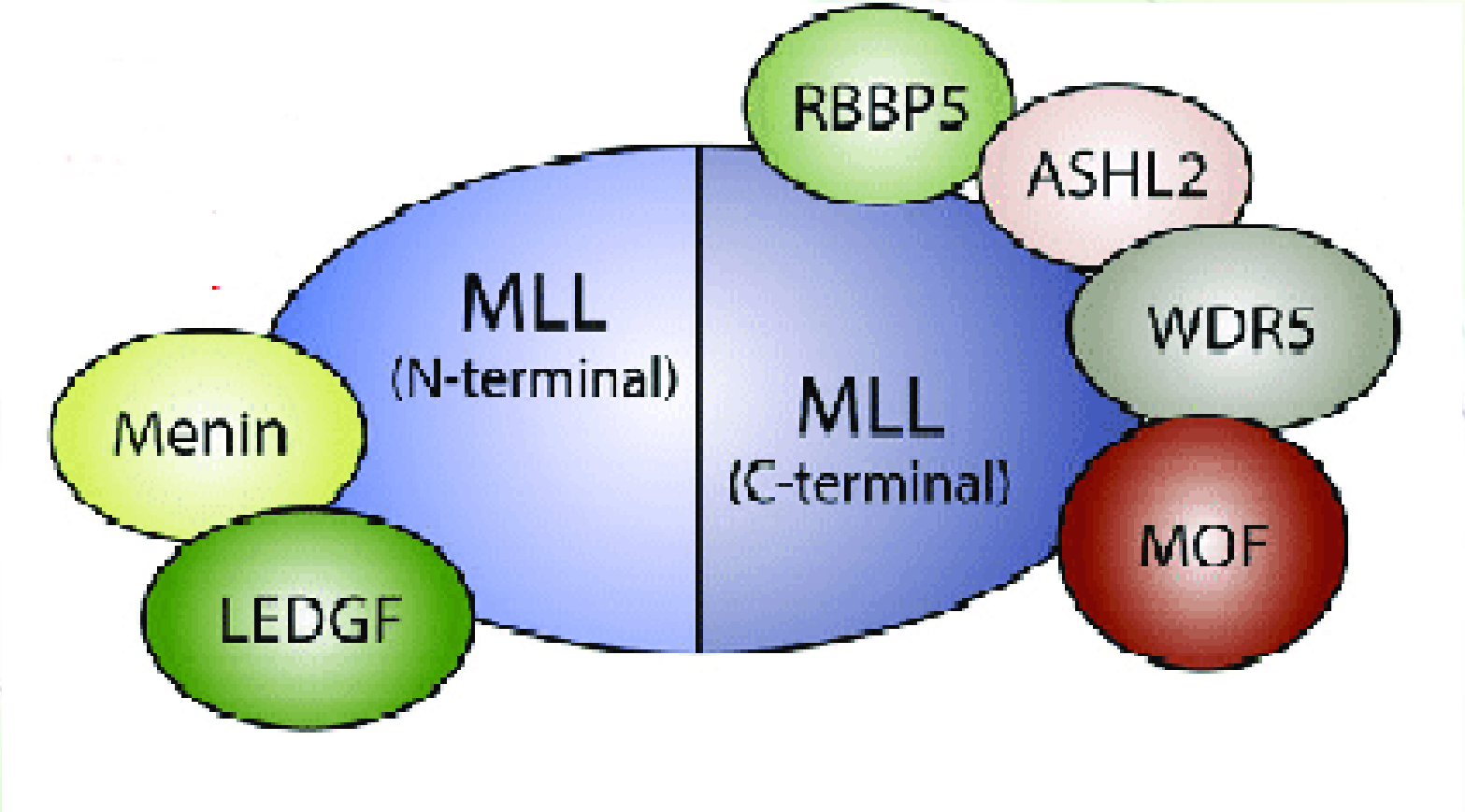




Protein-protein interaction

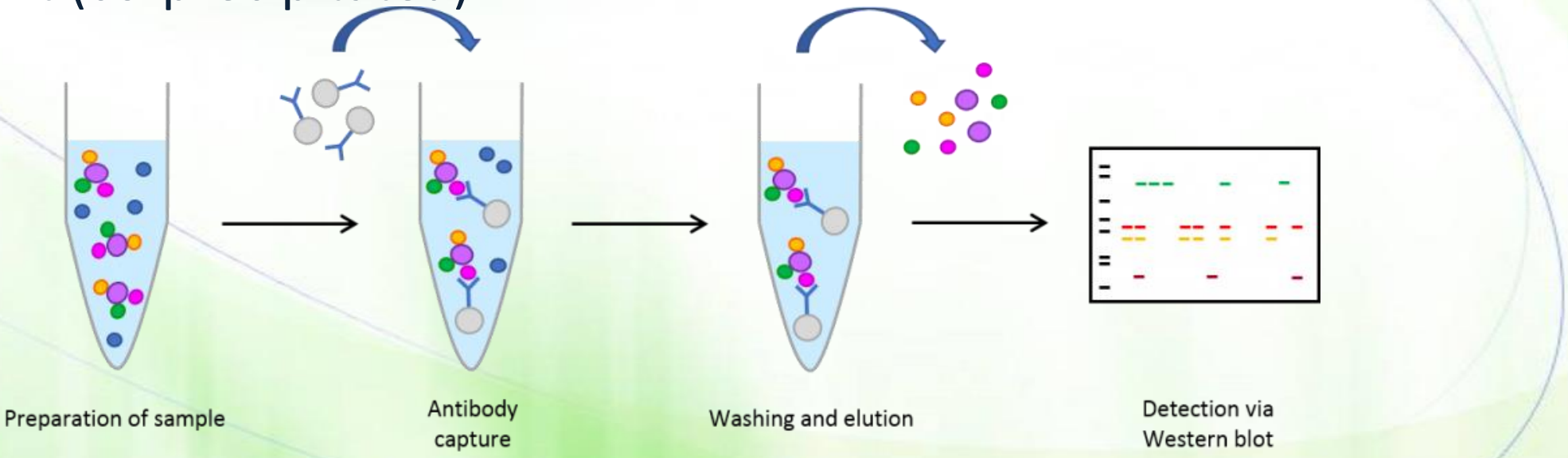
Co-immunoprecipitation

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).



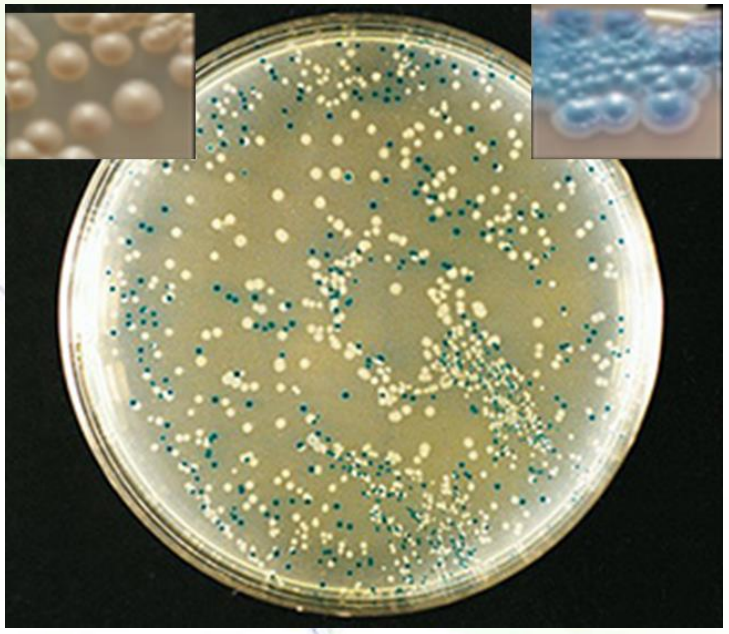
Protein-protein interaction

Yeast two-hybrid system

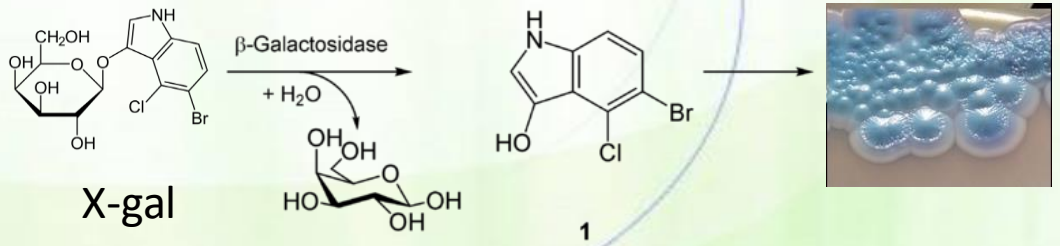
starting from a cDNA library

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

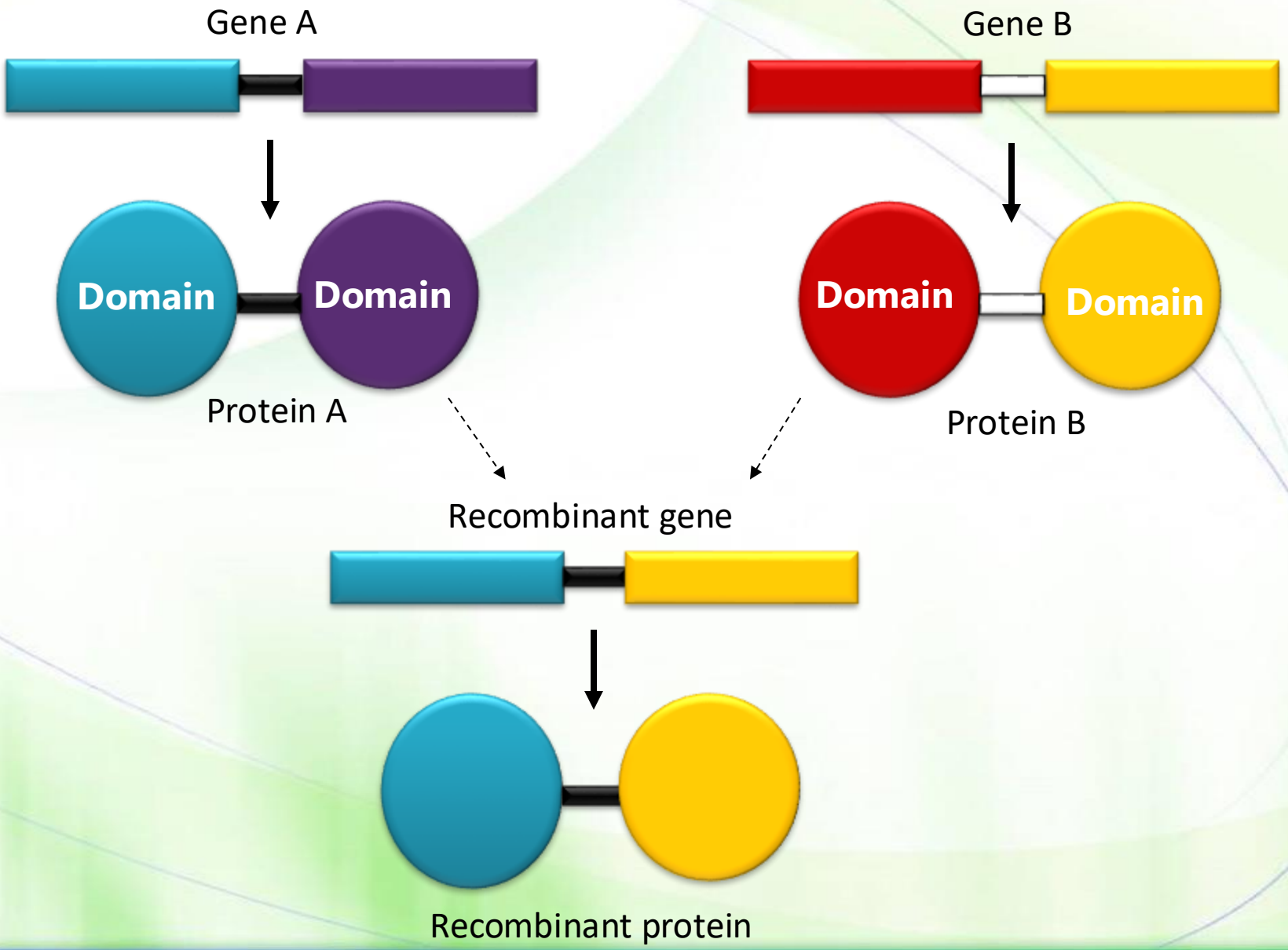
- To test if a protein interacts with another protein, a genetic system is used on yeast cells.
- The yeast cells are allowed to express the bacterial β-galactosidase , whose gene is under control of the gal4 transcription factor.
- The gal4 protein has two domains, a DNA-binding (DB) domain and an activation domain (AD).
- Yeast cells are grown in the presence of a lactose analog called X-gal, which generates a blue product when cleaved.
- When the β-galactosidase gene is activated, beta-galactosidase is produced, which cleaves X-gal generating blue colonies.



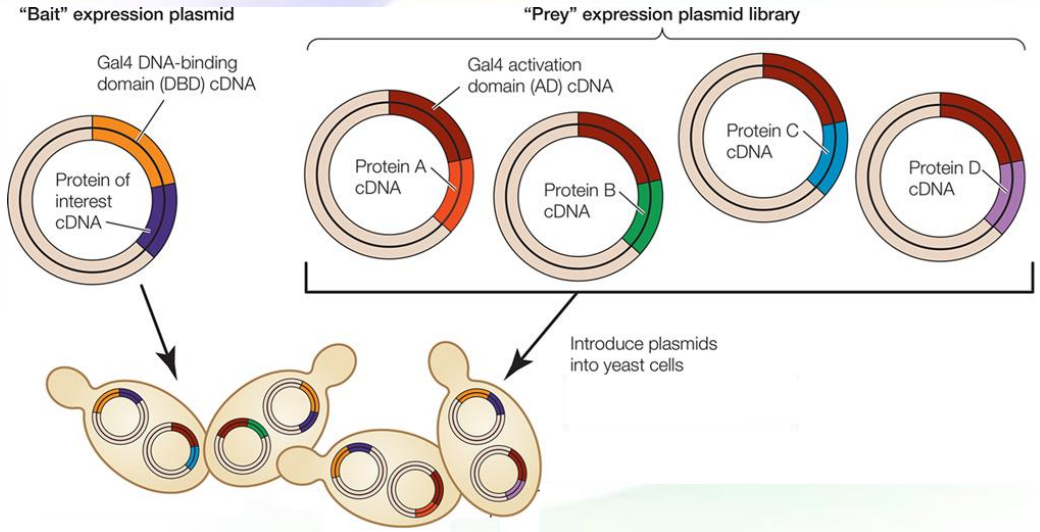
A. Regular transcription of the reporter gene

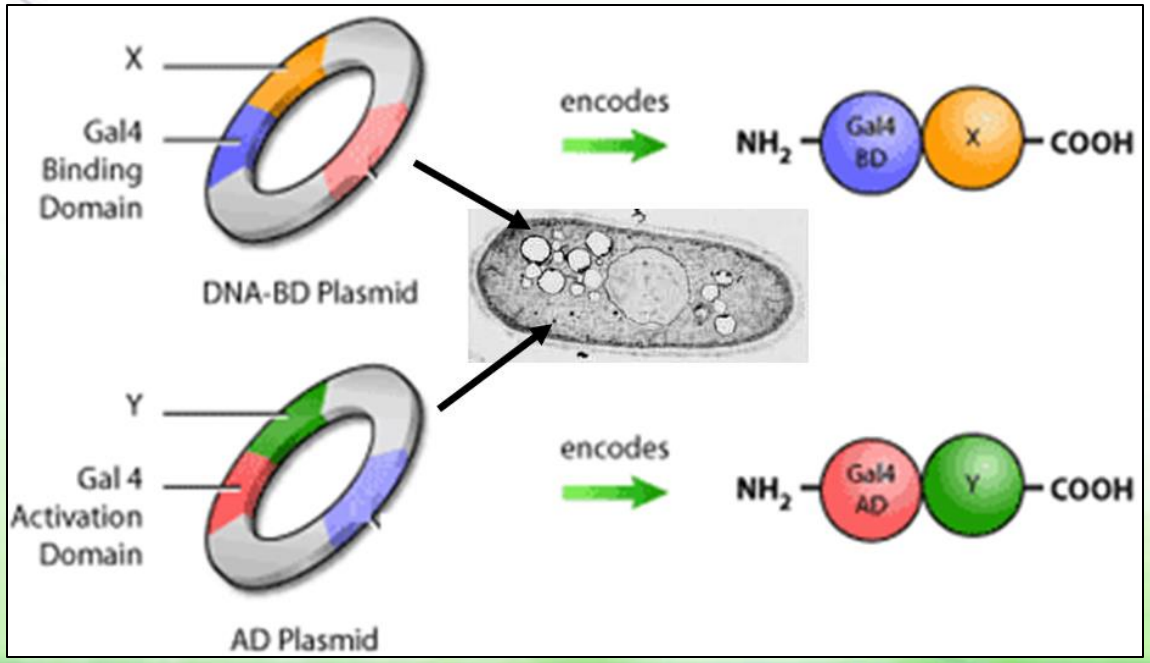


Production of a recombinant protein



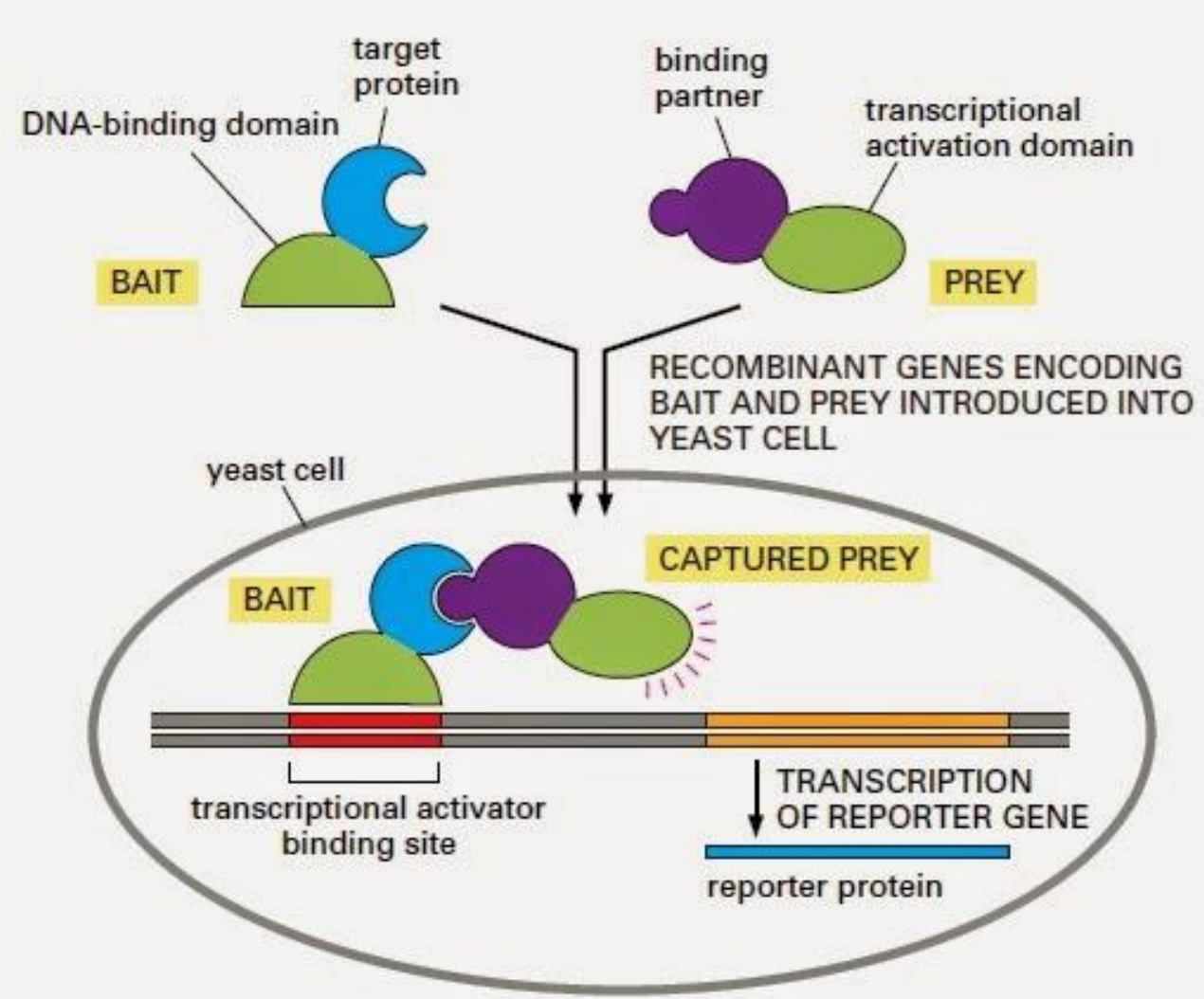
Cloning of hybrid proteins





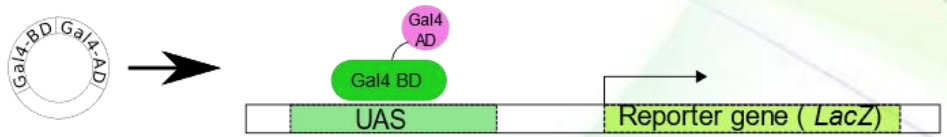
- To identify the unknown proteins (Y's) that interact with a known protein (X), the X gene is cloned so it is produced recombined with the DNA binding (DB) domain, and the unknown Y gene is separately cloned so that it produced recombined with the activation domain (AD).
  - A cDNA library can be created of multiple genes (cDNAs).
- Both recombinant plasmids are transferred into yeast cells so <u>all</u> cells express the known X -BD hybrid gene, but <u>each cell</u> expresses a different unknown Y-AD hybrid gene.

Quick illustration

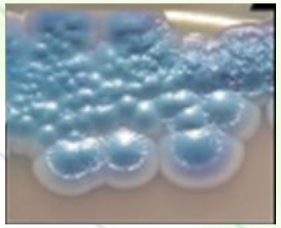


Yeast Two Hybrid System

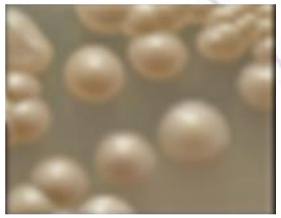
The possibilities and outcomes

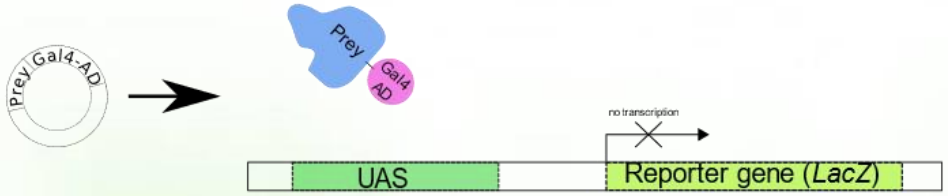


A. Regular transcription of the reporter gene

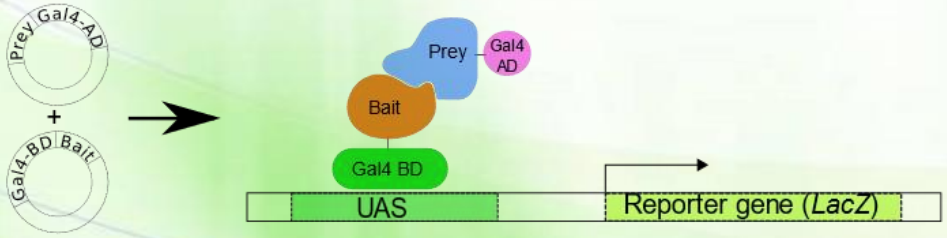


B. One fusion protein only (Gal4-BD + Bait) - no transcription

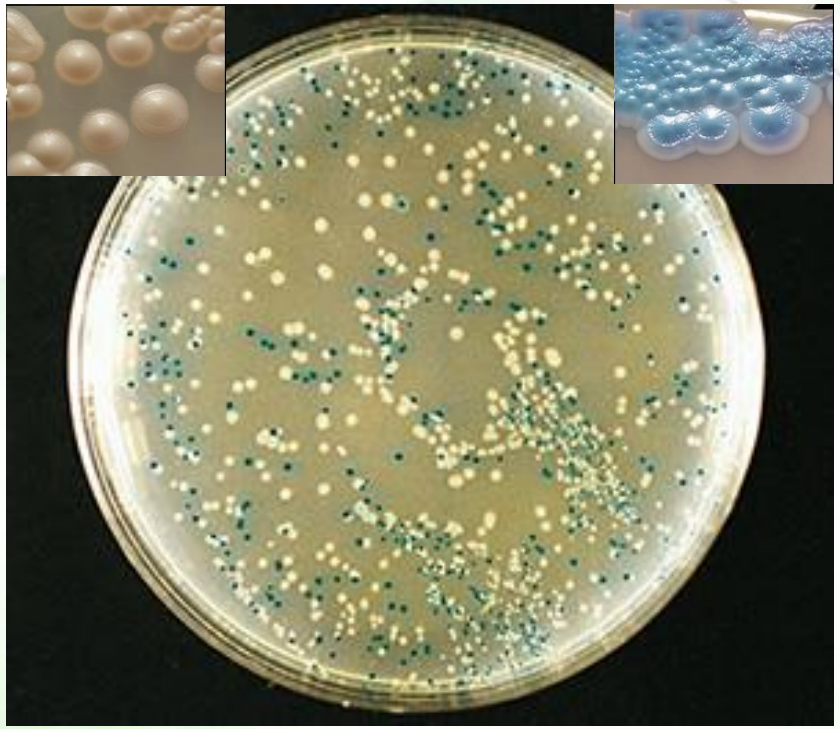




C. One fusion protein only (Gal4-AD + Prey) - no transcription



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.

The procedure

- A cDNA encoding a protein-of-interest is cloned into an expression plasmid adjacent to a cDNA encoding a DNA-binding domain (DBD) of a transcription factor (e.g., Gal4), yielding a DBD-fusion protein when expressed in cells.
- This plasmid is introduced into all yeast cells.
- A library of cDNAs is cloned into expression plasmids adjacent to a cDNA encoding a transcription factor activation domain (AD), yielding AD-fusion proteins when expressed in cells.
- The plasmids are introduced into the same yeast cells so that each one will have one.
- Protein–protein interactions between DBD- and AD-fusion proteins.
- The cells are grown on plates containing X-gal and each cell form a colony.
- If colonies turn blue, there is interaction.
- If colonies stay white, there is no interaction.