

YEAST TWO HYBRID SYSTEM :

Definition:

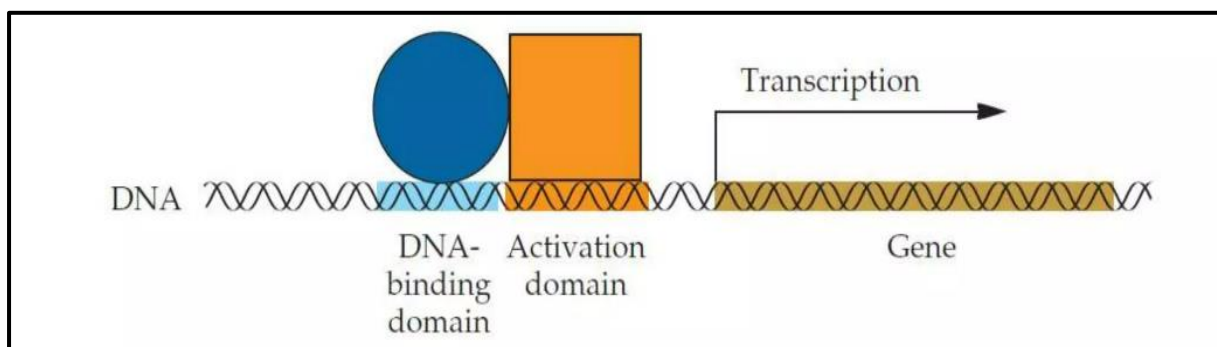
- Also known as Y2H or two hybrid screening.
- Molecular biology technique.
- Used to discover protein-protein interactions and protein-DNA interactions.
- Testing for physical interactions (such as binding) between two proteins or a single protein and a DNA molecular , respectively.

History:

- Pioneered by Stanley Fields and Ok-Kyu Song in 1989.
- Originally designed to detect protein-protein interactions using the Gal4 transcriptional activator of yeast *Saccharomyces Cerevisiae*.
- The Gal4 protein activated transcription of a gene involved in galactose utilization , which formed the basis of selection.
- Since then , the same principle has been adapted to describe many alternative methods, including some that detect protein-DNA interactions or DNA -DNA interactions, as well as methods that use different host organisms such as *Escherichia coli* or mammalian cells instead of yeast.

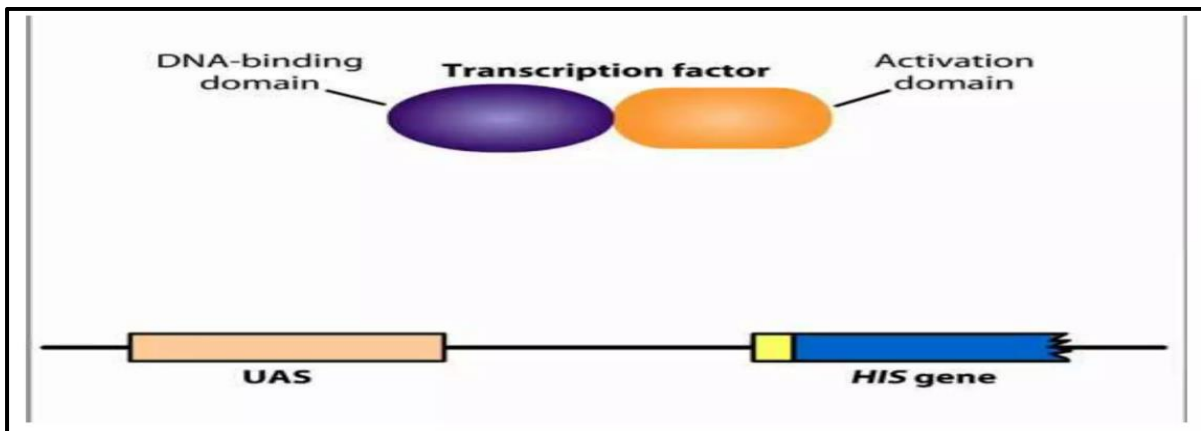
Principle:

- The Y2H assay relies on the expression of a reporter gene (such as lac Z or GFP) , which is activated by binding of a particular transcription factor.
- Transcription factor are structurally composed domains:
 1. The DNA-binding domain (BD), which binds DNA sequences.
 2. The activation domain (AD) which is responsible for recruitment of the transcription machinery.
- The query protein of interest fused with the BD is known as Bait and the protein library fused with AD is referred to as Prey.
- In order to activate reporter gene expression , a transcriptional unit must be present at the gene locus, which is only possible if Bait and Prey interact.

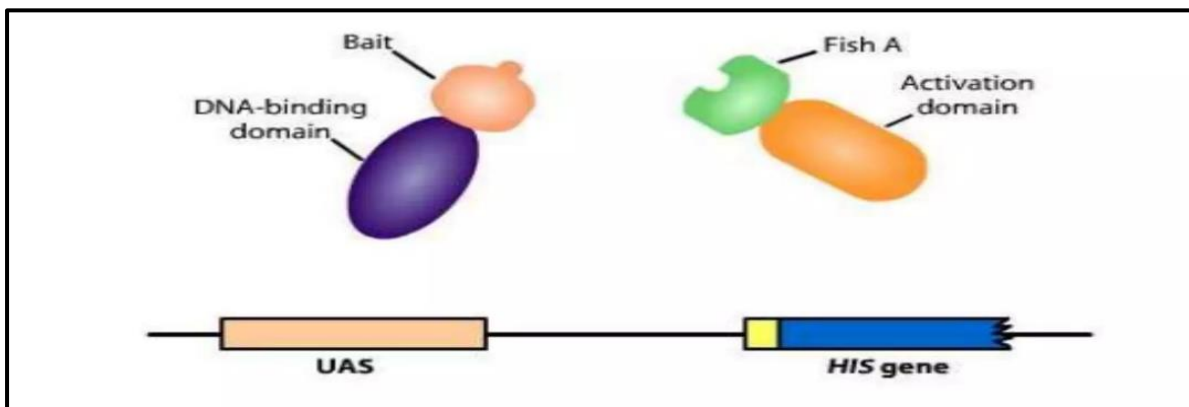


Yeast two hybrid protocol:

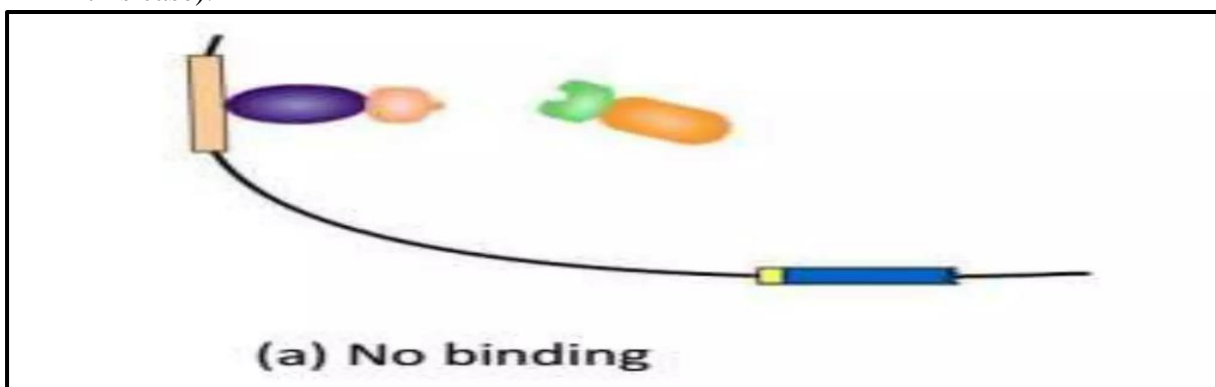
- The early yeast two hybrid systems were based on the finding that many eukaryotic transcription factors have separable DNA binding transcriptional activator domains.



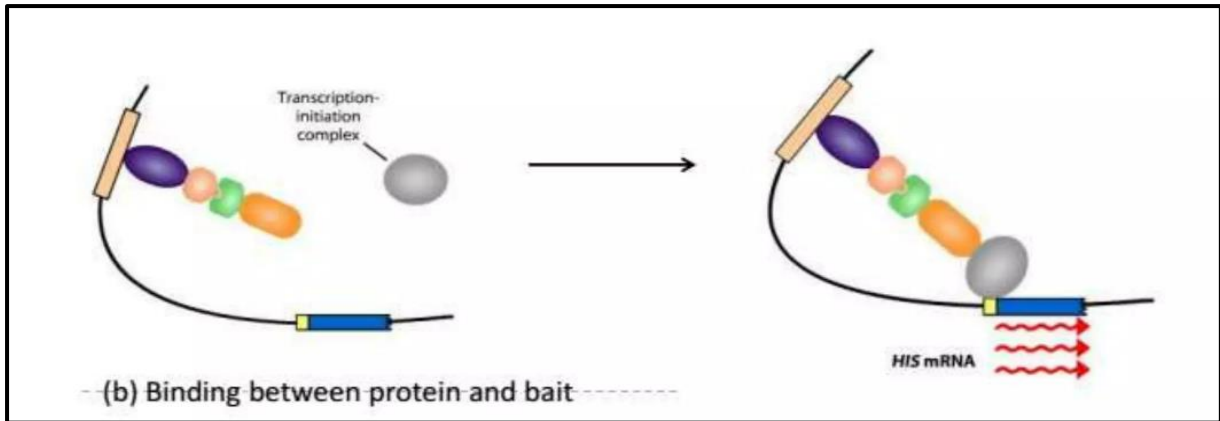
- The protein of interest, “bait”, is fused to a DNA-binding domain.
- Proteins that bind to bait, the “fish or Prey” are fused to a transcription activation domain.



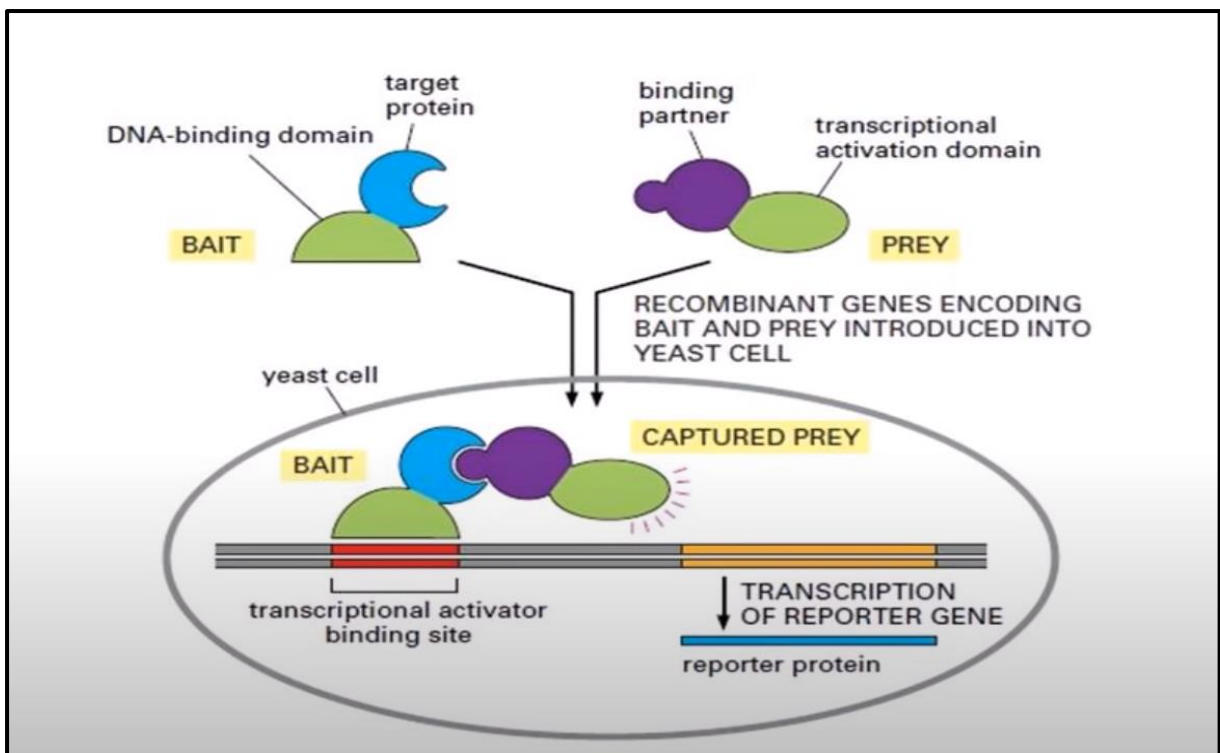
- Proteins that do not bind to the bait will not activate transcription of the reporter gene (HIS in this case).



- Any protein that binds to bait will activate the transcription of the reporter gene.



Yeast protein hybrid system:



Application:

- Determination of sequences crucial for interaction.
- Drug and poison discovery.
- Identifies novel protein protein interactions.
- Can identify protein cascades.
- Identifies mutations that affect protein-protein binding.
- Can identify interfering proteins in known interactions (Reverse Two Hybrid System).

Advantages:

- Immediate availability of cloned genes of interacting protein.
- Only a single plasmid construction is required .
- Interactions are detected in vivo.

- Weak, transient interactions can be detected.
- Can accumulate a weak signal over time.
- Protein purification is not necessary.
- No antibodies required.

Disadvantages:

- False positives are the largest problem with yeast two-hybrid systems.
- Possible incorrect protein folding in yeast.
- Gene encoding target protein must be available.
- Failed to detect some known interactions.
- Fusion proteins may inhibit original interactions.

Examples of uses of yeast two hybrid system:

- Identification of caspase substrates.
- Interaction of Calmodulin and L- Isoaspartyl Methyltransferase.
- Genetic characterization of mutations in E2E1.
- Peptide hormone receptor interactions.