

Chapter One: Yeast Two-hybrid System

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Yeast two-hybrid system overview

The yeast two-hybrid system is based on the fact that eukaryotic transcriptional activators consist of two individual domains, the DNA binding domain (DBD) and the activation domain (AD). The DBD recognizes a specific DNA sequence. The AD coordinates the assembly of the elements required for transcription and enables RNA polymerase II to transcribe a specific reporter gene downstream of the DBD domain.

Using the yeast two-hybrid system the protein of interest (X) is expressed as a fusion protein to the DBD (DBD-X; also known as the “bait” protein) and the activation domain is fused to the second protein of interest (Y), (AD-Y; also known as the “prey” protein).

The AD-Y fusion vector is introduced into a yeast strain containing the DBD-X fusion partner by transformation or mating. Only if proteins X and Y physically interact with one another are the DBD and AD brought together to activate expression of the downstream reporter gene (Figure 1).

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YEAST TWO-HYBRID SYSTEM



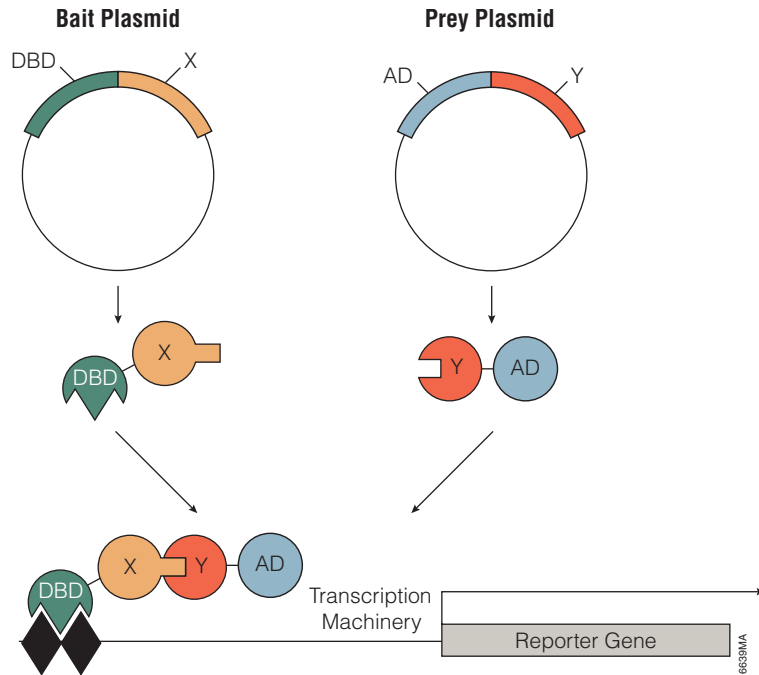


Figure 1. Principle of the yeast two-hybrid system. The protein coding sequence of the bait protein is cloned into a vector containing the DNA binding sequence (DBD-X (bait) fusion). The protein coding sequence of the prey protein is cloned into a vector that contains sequences for transcription activation (AD-Y (prey) fusion). Both vectors must also contain the necessary elements for growth and protein expression in yeast. The recombinant vectors are introduced into the appropriate yeast strain. Only if proteins X and Y physically interact with one another are the DBD and AD brought together to reconstitute a functionally active factor that binds to upstream specific sequences of the reporter gene and activates expression.

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Yeast two-hybrid system formats

There are several varieties of the yeast two-hybrid system. The two most commonly used systems differ in the nature of the DBD used to express the bait fusion protein (GAL4 or LexA), and the AD used to generate the prey fusion protein (GAL4, VP16 or B42).

In order to reduce the occurrence of false positives, typically a combination of four reporter genes (e.g., HIS3, URA3, ADE2 and *lacZ*) are stably integrated in single-copy numbers at different loci in the appropriate yeast genome. Induction of the HIS3, ADE2 or URA3 reporter genes allows monitoring of the transcription activation by growth on plates lacking histidine, adenine or uracil. Induction of the downstream *lacZ* gene results in a blue color yeast colony when assayed with X-Gal (5-bromo-4-chloro-3-indolyl-D-galactopyranoside).

When to use the yeast two-hybrid system

The yeast two-hybrid system is often the first method used to identify protein interactions. It provides an ideal format for screening one individual bait protein against large prey cDNA libraries, which can be generated by the user or purchased from commercial sources. Once tentative partners have been identified other methods are used to confirm and characterize the pair.

Primary reagent requirements for the yeast two-hybrid system

- Yeast expression vector containing DNA binding domain (e.g., GAL4) and sequence coding for the bait protein
- Yeast expression vector containing activator domain (e.g., VP16) and sequence coding for the prey protein
- Appropriate yeast strains
- Appropriate media for yeast growth and detection of interactions

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