Easily Identify and Characterize Protein-DNA Interactions with Our New Yeast One-Hybrid System

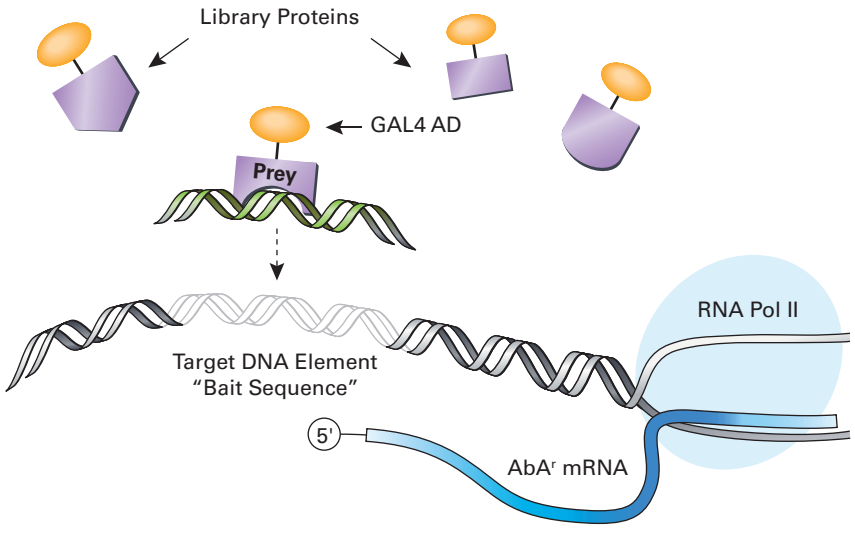
- **Highest performing yeast one-hybrid system**
- **Aureobasidin A selection eliminates background**
- **Complete system for easy construction and screening of cDNA libraries directly in yeast**

Clontech's **Matchmaker™ Gold Yeast One-Hybrid Library Screening System** provides a simple and efficient method for identifying and characterizing novel protein-DNA interactions. The system uses SMART™ cDNA synthesis technology, which allows cDNA libraries to be created from any tissue source, starting with as little as 100 ng of total RNA. It also employs **Aureobasidin A** (AbA; 1) selection, which provides the most stringent yeast one-hybrid (Y1H) screening strategy available.

The System

In the Matchmaker Gold Yeast One-Hybrid Library Screening System, 1–3 copies of your target DNA sequence (i.e., the bait) are cloned into the reporter vector pAbAi. The resulting pBait-AbAi construct is then integrated into the genome of the Y1HGold yeast strain by homologous recombination to generate a bait-specific reporter strain.

A cDNA library expressing potential DNA-binding proteins (i.e., prey) as fusions to the GAL4 transcription activation domain (AD) is constructed directly in the pBait-AbAi reporter strain. When a prey protein binds to the DNA target sequence (see figure below), transcription of the Aureobasidin A resistance gene (AbA^r) is activated, allowing the cell to grow on medium containing the antibiotic Aureobasidin A (AbA). In library screens, the plasmids encoding the library-derived prey proteins can be rescued from the surviving yeast clones and subjected to further analysis.



Screening for protein-DNA interactions with the Matchmaker Gold Yeast One-Hybrid System.

SMART Technology

The cDNA inserts for the prey library are created by SMART cDNA synthesis, which results in the incorporation of known primer sequences at both ends of the cDNA. Consequently, SMART-generated cDNA:

- *is available for amplification by PCR*—allowing the construction of libraries from nanogram amounts of starting RNA.
- *is flanked by sequences that are homologous to the cloning site of the linearized library vector, pGADT7-Rec*—allowing homologous recombination between the cDNA and the pGADT7-Rec vector upon transformation into the bait-specific reporter strain (Figure 1).

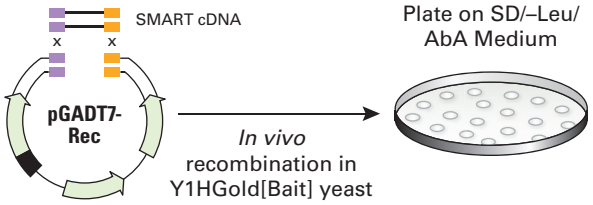


Figure 1. Use SMART technology and yeast biology to construct and screen your library. Your library is simultaneously constructed and screened directly in yeast. First, SMART cDNA synthesis technology is used to create a pool of cDNA that is flanked by sequences homologous to the ends of the linearized pGADT7-Rec vector. Next, the newly created Y1HGold-Bait reporter strain is transformed with the cDNA pool and pGADT7-Rec, which undergo homologous recombination within yeast. The yeast cells are then plated on SD/–Leu/+AbA to select for colonies that contain an active reporter (i.e., positive Y1H interactions).

Get Screening Results Fast!

With the Matchmaker Gold Yeast One-Hybrid Library Screening System, one-hybrid screening can be accomplished quickly and easily with the following steps:

Step 1. Create a bait construct by cloning 1–3 copies of the target DNA-binding sequence into pAbAi.

Step 2. Create a bait-specific reporter strain by transforming and integrating the linearized pBait-AbAi construct into the Y1HGold yeast strain and selecting on SD/–Ura medium, available in our **Yeast Media Set 1 Plus.**

Step 3. Confirm the integration of the bait sequence by colony PCR using **Matchmaker Insert Check PCR Mix 1.**

Step 4. Use SMART technology to synthesize cDNA that is flanked by sequences that are homologous to the ends of the linearized pGADT7-Rec vector.

Step 5. *Create and screen your Y1H library in a single step:* Cotransform your bait-specific Y1HGold reporter strain with the SMART-generated cDNA and pGADT7-Rec vector, and plate on SD/–Leu/+AbA.

Step 6. Harvest the resulting colonies, which contain putative DNA-binding proteins, and analyze further (e.g., with the **Matchmaker Insert Check PCR Mix 2** and the **Easy Yeast Plasmid Isolation Kit**).

The Matchmaker Gold Yeast One-Hybrid Library Screening System is the most convenient and advanced Y1H screening tool available, allowing library screens to be accomplished in less time and with greater confidence than ever before.

Aureobasidin A Selection Eliminates Background

Matchmaker Gold Systems are unique because they use the AbA^r gene as a novel reporter that confers resistance to AbA, a potent antifungal agent that is toxic to *S. cerevisiae.* Selecting for resistance to this highly stable depsipeptide makes Y1H library screening very straightforward (Figure 1), as AbA effectively kills yeast cells that are not expressing the AbA^r reporter. Aureobasidin A and all of the required media are supplied in our **Yeast Media Set 1 Plus.**

Reference

1. Takesako, K. *et al.* (1991) *J. Antibiot.* **44**(9):919–924.

Ordering Information			
Product	**Size**	**Cat. No.**	
Matchmaker Gold Yeast One-Hybrid Library Screening System	5 rxns	630491	NEW!
Yeast Media Set 1 Plus	each	630493	NEW!
Matchmaker Insert Check PCR Mix 1	100 rxns	630496	NEW!
Matchmaker Insert Check PCR Mix 2	100 rxns	630497	NEW!
Easy Yeast Plasmid Isolation Kit	50 preps	630467	NEW!

Notice to Purchaser

Please see the HotStart Antibody, PCR, PCR Polymerase, SMART™ Amplification Products, and Aureobasidin Resistance Gene licensing statements at **www.clontech.com/licensing**