Matchmaker™ Gold Yeast Two-Hybrid System

A novel reporter system allows you to discover interactions more easily and with fewer false positives

- **4 reporters & 3 promoters lead to fewer false positives**
- **Interacting fusion proteins produce resistance to Aureobasidin A—a very potent yeast antibiotic**
- **Antibiotic, nutritional, and blue/white selection for simple yet stringent screens**

Clontech's Matchmaker Systems are highly advanced tools for identifying and characterizing novel protein-protein interactions (PPIs). Our latest and most powerful incarnation, the **Matchmaker Gold Yeast Two-Hybrid System,** adds a sensitive **Aureobasidin A** (AbA; 1) antibiotic resistance marker to two nutritional reporters and blue/white color selection to create a four-reporter system with the easiest, most stringent yeast two-hybrid (Y2H) screening strategy available (Figure 1).

Aureobasidin A effectively kills yeast, but when the *AUR1-C* gene is turned on by a positive interaction between GAL4-hybrid proteins, the yeast become AbA-resistant. Secondary confirmation of positive clones employs four reporters regulated by three different GAL4-reponsive promoters. This effectively eliminates false positives, and leaves you with greater numbers of genuine positives. Quality screening results like these, as well as our simple "Mate & Plate™" library screening protocol, save you time and make your search for PPIs faster, easier, and more fruitful.

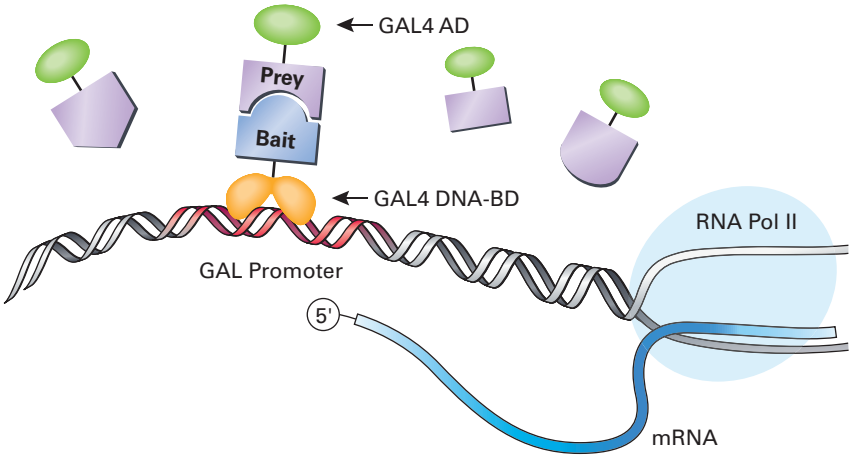

Figure 1. Yeast two-hybrid system design. Library-derived, transcription-activating prey fusion proteins that interact with the DNA-binding bait fusion protein activate the expression of reporter genes.

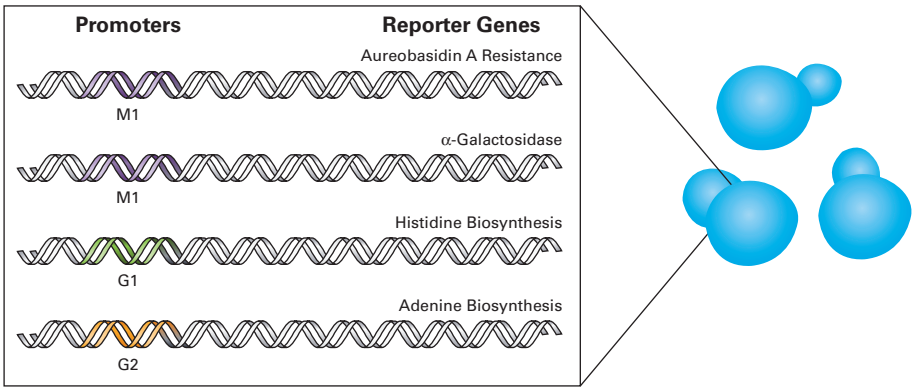

Figure 2. Four reporters give Matchmaker Gold its high stringency. Interacting bait and prey fusion proteins drive the expression of four different reporters from three different GAL4-responsive promoters (M1, G1, and G2), which are stably integrated in the genome of the reporter strain, Y2HGold. Aureobasidin A (AbA) resistance and the two auxotrophic reporters for histidine and adenine biosynthesis confer growth selection in the presence of AbA and on histidine- and adenine-deficient media, while the α-galactosidase reporter produces blue colonies in the presence of X-alpha-Gal.

Two-Hybrid System Principles

Y2H systems exploit the modular nature of eukaryotic transcription factors, which consist of a sequence-specific DNA-binding domain (DNA-BD) and an RNA Pol II-recruiting transcription activation domain (AD; 2, 3). In Matchmaker Systems, a known protein of interest is fused to the DNA-BD of the yeast GAL4 transcription factor to create a "bait" protein. Interacting partner proteins, often derived from a library, are expressed as fusions to the AD of yeast GAL4, to create "prey" proteins (Figure 1). When pairs of interacting bait and prey fusion proteins are coexpressed in a yeast cell, GAL4 function is restored and the interacting fusion proteins are able to activate transcription of the reporter genes. In Matchmaker Gold, yeast clones that harbor interacting protein pairs can then be identified by the presence of the four reporters (Figure 2). In library screens, the plasmids containing the coding sequences for the library-derived prey proteins can be rescued from the surviving yeast clones, and subjected to further analysis and sequencing.

Aureobasidin A Selection Eliminates Background

Matchmaker Gold Systems are unique because they use the *AUR1-C* gene as a novel reporter that confers resistance to AbA, a potent antibiotic toxic to *S. cerevisiae.* Resistance to this highly stable antifungal depsipeptide (see page 9) allows straightforward Y2H library screening to be achieved without the optimization required when using nutritional selection alone. *HIS3*-

Matchmaker™ Gold Yeast Two-Hybrid System...continued

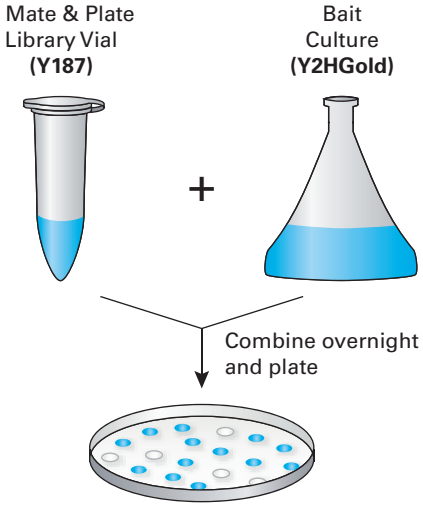

Figure 3. The Mate & Plate Protocol. To screen a Matchmaker Mate & Plate Library, an aliquot of the library in the Y187 strain (*MATα*) is simply mixed with a bait-expressing culture of the Y2HGold strain (*MATa*). The mated strains are cultured overnight and plated on selective agar medium (e.g. -Leu/-Trp + AbA + X-alpha-Gal).

based selection can produce high numbers of background colonies in primary screens, whereas AbA very effectively kills yeast cells not expressing the *AUR1-C* reporter. As a result, even low stringency primary screens using AbA are quite definitive and produce a high percentage of genuinely positive clones, without the interference of background colonies.

Four Reporters Identify Genuine Positives

The stringency of Matchmaker Gold lies in the use of four selective reporter genes: *AUR1-C, HIS3, ADE2,* and *MEL1* (α-galactosidase), whose expression is driven by 3 different GAL4-binding promoters (Figure 2). All of the reporter genes are stably integrated into the genome of the **Y2HGold** reporter strain. This strategic combination of four reporters virtually eliminates false positives, especially those arising from spurious GAL4 promoter-binding prey proteins, which might bind one promoter sequence but not all three.

Mate & Plate Libraries & Screening

Another major advantage of Matchmaker Gold is that we've replaced cumbersome library-scale yeast transformation with an easy "Mate & Plate" strategy that consists of combining the two haploid yeast strains that independently express the bait and prey fusion proteins (Figure 3). **Y2HGold** is a *MATa* strain which harbors the four integrated reporter genes and is transformed with a pGBKT7 plasmid to express the bait protein. The library "prey" strain, **Y187,** is a *MATα* strain and the ideal mating partner for Y2HGold.

Clontech offers a variety of pretransformed **Mate and Plate™ Libraries** in Y187 (see pages 6–7). Alternatively, users can either create and transform their own library using the **Make Your Own "Mate & Plate" Library System** (see pages 4–5), or transform a plasmid expressing a user-selected prey fusion protein. Combining the Y2HGold and Y187 strains for mating is very easy and results in diploid yeast that coexpress the desired combinations of the bait and prey proteins.

High Numbers of Confirmed Positive Clones

Due to the strong selective power of AbA resistance, first-round, low stringency screening selects only for blue, AbA-resistant colonies. Two-thirds of these putative positive clones are later confirmed by high stringency screening, which selects for all four reporters. As a result, we now recommend low stringency screening to generate a large pool of colonies, followed by high stringency verification. This leads to more genuine positives and fewer false positives (Figure 4).

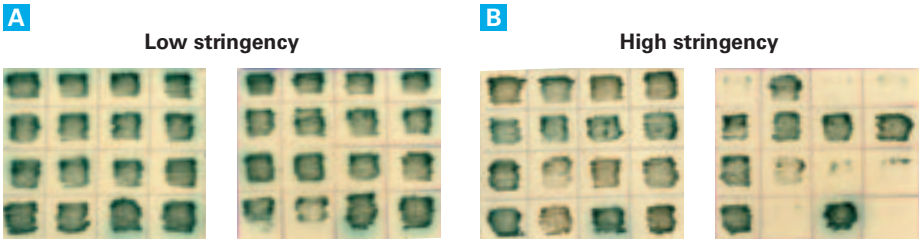

Figure 4. Secondary Matchmaker Gold screening confirms high numbers of positive clones. A Y2HGold bait containing the POU domain from the mouse Oct4 transcription factor (BD-POU_{mOct4}) was used to screen the Mate & Plate Universal Mouse (Normalized) Library for Oct4-binding proteins. 32 colonies from a low stringency primary screen (DDO + AbA, 60 ng/ml + X-alpha-Gal) were selected and replated/patched onto fresh low stringency medium (**Panel A**) and onto high stringency medium (QDO + AbA, 60 ng/ml + X-alpha-Gal) (**Panel B**) to confirm the expression of all four Matchmaker Gold reporters. Of the 32 originally selected colonies, 25 were confirmed positive for the 4 reporters. DDO = Double dropout medium: SD/–Leu/–Trp. QDO = Quadruple dropout medium: SD/–Ade/–His/–Leu/–Trp.

Table I: Mouse Oct4-Binding Proteins Identified in Matchmaker Gold Library Screening[1]

Protein	Function
E2I/Ubc9	Small ubiquitin-related modifier (SUMO) enzyme involved in sumoylation of Oct4; regulates Oct4 stability
PIAS1	An E3 ligase protein inhibitor of activated STAT1; a potent inhibitor of Oct4-mediated transcriptional activation
PSMB5	Proteasome beta5 subunit; may mediate the interaction of Oct4 with proteasomes, which regulate cellular processes through protein degradation.

1 Mate & Plate Library - Universal Mouse (Normalized).

Matchmaker™ Gold Yeast Two-Hybrid System...continued

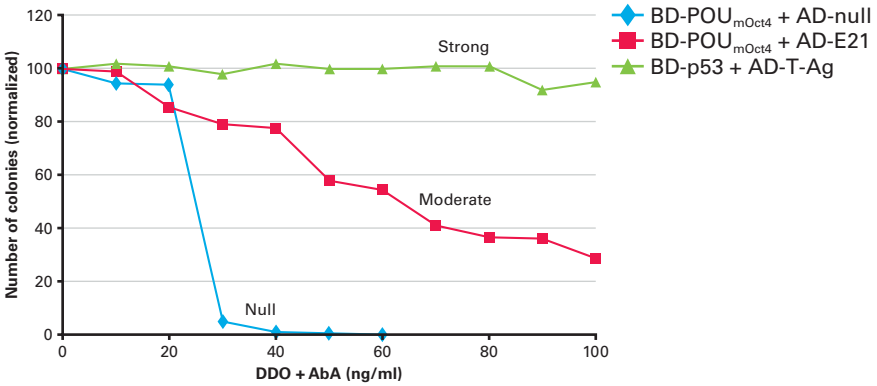

Figure 5. Titration of Aureobasidin A for three different Matchmaker protein pairs. Y2HGold yeast clones coexpressing one of three bait/prey fusion protein pairs: a negative control (BD-POU$_{mOct4}$ + AD-null); a pair of putative interactors (BD-POU$_{mOct4}$ + AD-E2I); or a positive control (BD-p53 + AD-T-Ag), were grown on DDO agar (SD/-Trp/-Leu) in the presence of increasing concentrations of AbA. The relative ability of each strain to grow in the presence of AbA reflects the strength of the interactions between the bait and prey proteins. Libraries are usually screened in the presence of AbA at 60 ng/ml, which demonstrates definitive selection for interacting hybrids. POU$_{mOct4}$ = POU domain from the mouse transcription factor, Oct4.

Aureobasidin A Resistance is Highly Selective

To demonstrate the highly selective properties of AbA resistance, AbA was titrated against three diploid Y2HGold clones that each expressed different bait/prey protein pairs (Figure 5). A negative control strain that coexpressed a non-interacting protein pair (BD-POU$_{mOct4}$ bait and an unmodified GAL4 AD protein, AD-null), was completely unable to grow in the presence of AbA concentrations above 40 ng/ml. When the BD-POU$_{mOct4}$ bait was paired with a putatively interacting prey protein (AD-E2I), colonies were able to grow in the presence of 70–100 ng/ml AbA. This result suggests that these proteins interact well enough to activate *AUR1-C* expression and allow colony growth on AbA medium. In a positive control, the strongly interacting protein pair of DNA-BD-p53 and AD-SV40 large T antigen enabled the vast majority of colonies to grow in the presence of 100 ng/ml AbA—the highest concentration tested, and indicated a very high level of *AUR1-C* expression.

Superior to Nutritional Selection Alone

The use of auxotrophic reporters alone for Y2H screening often requires optimization steps, especially in the case of *HIS3*-based selection, to ensure that the growth conditions are sufficiently selective. The leakiness of *HIS3* selection necessitates the use of 3-AT (a competitive inhibitor of the His3 protein), which must be titrated and included in the growth medium to suppress the growth of background colonies. With AbA selection and the two nutritional markers of Matchmaker Gold, growth selection is sufficiently stringent so as to make the use of 3-AT unnecessary.

With the Matchmaker Gold System and a wide selection of tissue-specific, normalized, and universal Mate & Plate Libraries, Clontech offers the most convenient and advanced Y2H screening tools available. You can even construct your own library with the Make Your Own "Mate & Plate" Library System. Library screens can now be accomplished in less time and with greater confidence than ever before.

Product	Size	Cat. No.
Matchmaker Gold Yeast Two-Hybrid System	each	630489
Mate & Plate Library – Universal Mouse (Normalized)	2 x 1 ml	630482
Make Your Own "Mate & Plate" Library System	5 rxns	630490
Yeast Media Set 2	each	630494
Yeast Media Set 2 Plus	each	630495
Aureobasidin A	1 mg	630466
X-alpha-Gal	100 mg 250 mg	630462 630463

Matchmaker™ Gold Yeast Two-Hybrid System Components

- pGBKT7 DNA-BD Cloning Vector
- pGADT7 AD Cloning Vector
- pGBKT7-53 Control Vector
- pGBKT7-Lam Control Vector
- pGADT7-T Control Vector
- Y2HGold Yeast Strain
- Y187 Yeast Strain
- YPDA Broth
- YPDA with Agar
- SD/-Trp with Agar
- SD/-Leu with Agar
- Yeastmaker™ Yeast Transformation System 2

Yeast Media Set 2 Components (0.5 L pouches)

- YPDA Broth (2 each)
- YPDA with Agar
- SD/-Leu Broth
- SD/-Leu with Agar
- SD/-Trp Broth
- SD/-Trp with Agar
- SD/-Leu/-Trp with Agar (DDO; 10 each)
- SD/-Ade/-His/-Leu/-Trp with Agar (QDO)

Yeast Media Set 2 Plus Components

- Yeast Media Set 2
- Aureobasidin A (1 mg)
- X-alpha-Gal (250 mg)

Notice to Purchaser

Please see the Aureobasidin Drug, Aureobasidin Resistance Gene, Matchmaker™ Two-Hybrid System, and Reverse Two-Hybrid Technology licensing statements on page 40.

References

1. Takesako, K. *et al.* (1991) *J. Antibiot.* **44**(9):919–924.
2. Fields, S & Song, O. (1989) *Nature* **340**(6230):245–246.
3. Chien, C. T. *et al.* (1991) *Proc. Nat. Acad. Sci. USA* **88**(21):9578–9582.