Yeast Two-Hybrid Systems

Protein-Protein &
Protein-DNA Interactions

Matchmaker™ Gold

Powerful products for characterizing your protein interactions

Clontech

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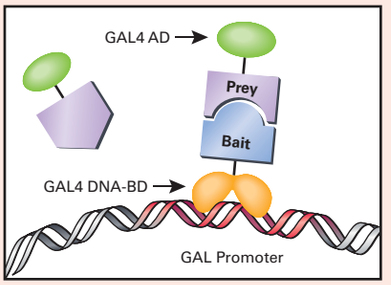

Less Background, Fewer False Positives, More Yeast Two-Hybrid Interactions

In the Matchmaker Gold Yeast Two-Hybrid System, resistance to a potent antifungal, Aureobasidin A (AbA), virtually eradicates background colonies from primary screens. Then, secondary confirmation of positive clones eliminates false positives by employing four reporters regulated by three different GAL4-responsive promoters. The result is a two-hybrid system of unparalleled stringency.

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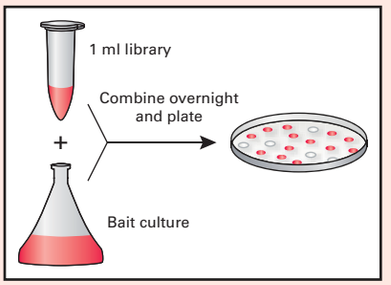

Just Mate & Plate—That's it!

Can you set up an overnight yeast culture? If so, then you can screen a yeast two-hybrid library. Clontech's Mate & Plate Libraries offer the simplest, most straight-forward method of screening a yeast two-hybrid library for interacting protein pairs. To screen these ready-to-go cDNA libraries simply:

1. Set up a mixed culture of your bait-expressing reporter strain and one vial of the Mate & Plate Library
2. Culture overnight
3. Plate the next day

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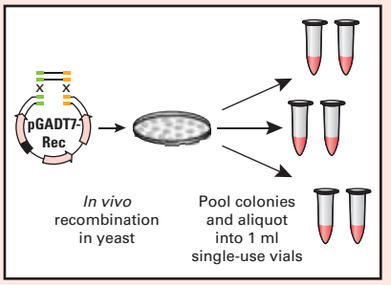

The Easiest Way to Make Your Own Two-Hybrid Library... By Far!

If our wide selection of Mate & Plate Libraries does not suit your needs, we offer the Make Your Own "Mate & Plate" Library System. The kit allows you to create library aliquots for hundreds of yeast two hybrid screens—in less than a week

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The Complete Solution!

Never used yeast before?

No need to worry; our Yeast Media Sets, transformation systems, and plasmid isolation kits make it easy for you.

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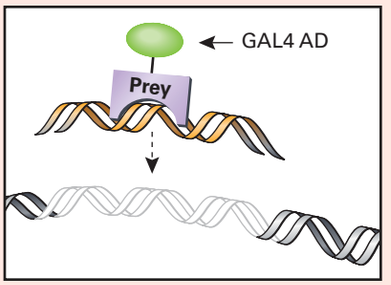

Matchmaker Gold for Protein-DNA interactions.

The highest-performing yeast one-hybrid system makes full use of our novel and highly stringent AbA reporter. Construct and screen a library simultaneously with our Matchmaker Gold Yeast One-Hybrid Library Screening System.

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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,** employs sensitive **Aureobasidin A** antibiotic resistance (1), 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).

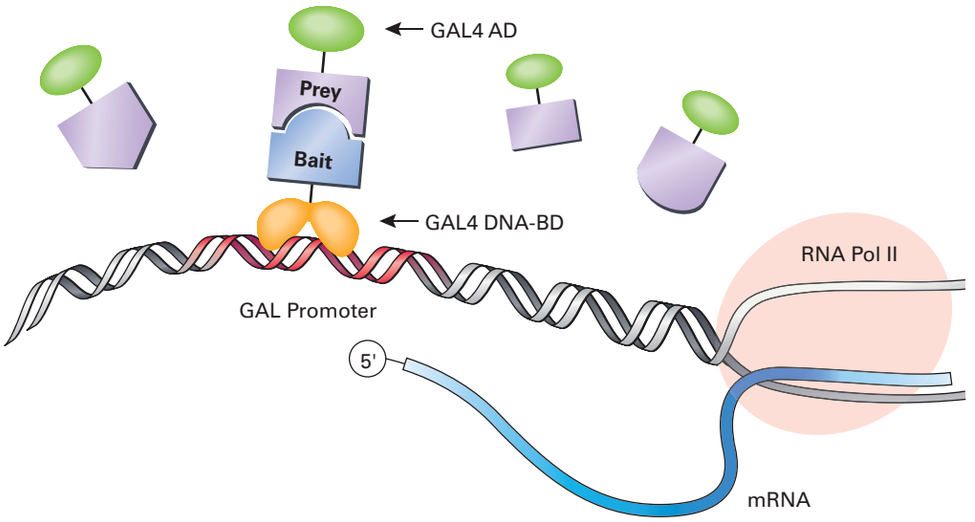

Figure 1. Yeast two-hybrid system design. Library-derived, transcription-activating prey fusion proteins that interact with the DNA-binding bait fusion protein activate the expression of reporter genes.

GAL4-Based Two-Hybrid Systems

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, the interacting fusion proteins are able to activate transcription of the reporter genes. In Matchmaker Gold, interacting protein pairs activate a suite of four reporter genes (Figure 2).

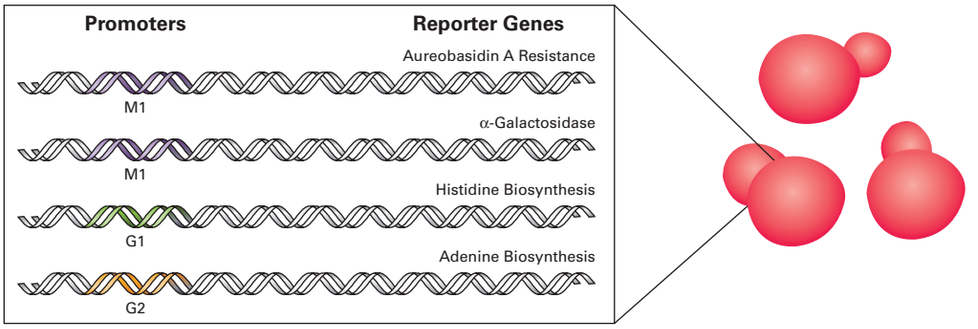

Figure 2. Four reporters give Matchmaker Gold its high stringency. Interacting bait and prey fusion proteins drive the expression of four different reporters from three different GAL4-responsive promoters (M1, G1, and G2), which are stably integrated in the genome of the reporter strain, Y2HGold. Aureobasidin 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.

Aureobasidin A Eliminates Background

What makes Matchmaker Gold Systems so unique is the use of a novel reporter that confers resistance to Aureobasidin A (AbA), which is a potent and lethal *S. cerevisiae* antibiotic. When positive bait-prey interactions occur in a yeast cell, the *AUR1-C* reporter gene is turned on and the cell is able to grow in the presence of AbA. Since AbA kills the yeast cells not expressing the *AUR1-C* reporter, background colonies never have a chance to grow, so even low-stringency primary screens are quite definitive and produce a high percentage of genuinely positive clones. Selecting for AbA resistance requires none of the optimization that is needed when nutritional markers are used alone, and produces screens without the interference from background colonies.

Four Reporters Identify Genuine Positives

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

Simplified Screening with Mate & Plate™ Libraries

Another major advantage of using the Matchmaker Gold System is that we've replaced cumbersome library handling and large-scale yeast transformation with a simple and easy "Mate & Plate™" strategy. This technique consists of combining two haploid yeast strains of opposite mating types, that independently express either the bait or prey fusion proteins (Figure 3). First, you create a Y2HGold bait reporter strain (which is of the *MATa* mating type) by transforming it with your pGBKT7-bait plasmid. Mate and Plate Libraries which express cDNA library prey proteins in a Y187 *MAT*α strain, are the ideal mating partners for Y2HGold.

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

Then, by simply combining your Y2HGold bait reporter strain with an aliquot of Mate & Plate Library in an overnight culture, diploid yeast clones arise which coexpress the desired combinations of bait and prey proteins. Clontech offers a variety of these pretransformed Mate and Plate Libraries, including tissue specific, normalized and universal libraries to provide an array of easy, ready-made Y2H screening options. Alternatively, you can create your own library using our convenient **Make Your Own "Mate & Plate™" Library System.**

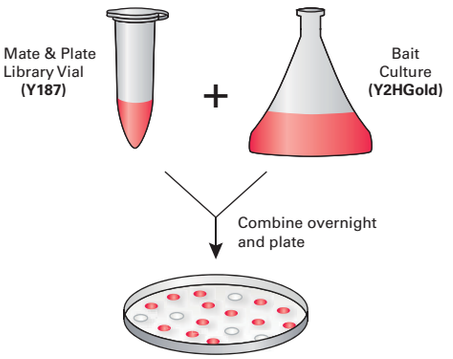

Figure 3. The Mate & Plate Protocol. To screen a Matchmaker Mate & Plate Library, an aliquot of the library in the Y187 strain (*MATα*) is simply mixed with a bait-expressing culture of the Y2HGold strain *(MATa)*. The mated strains are cultured overnight and plated on selective agar medium containing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

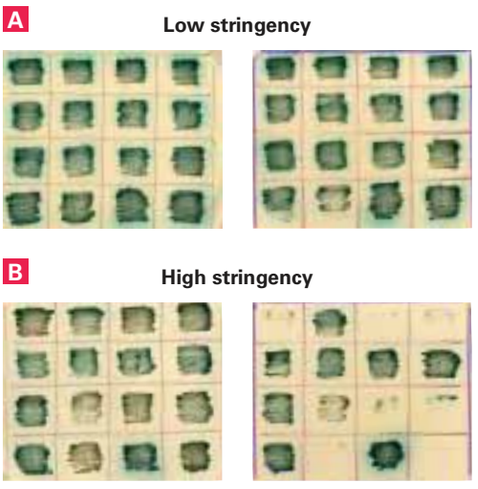

Figure 4. Secondary Matchmaker Gold screening confirms high numbers of positive clones. A Y2HGold bait containing the POU domain from the mouse Oct4 transcription factor (BD-POU$_{mOct4}$) was used to screen the Mate & Plate Universal Mouse (Normalized) Library for Oct4-binding proteins. 32 colonies from a low stringency primary screen (DDO + AbA + X-alpha-Gal) were selected and replated/patched onto fresh low stringency medium (**Panel A**) and also onto high stringency medium (QDO + AbA + X-alpha-Gal) (**Panel B**) to confirm the expression of all four Matchmaker Gold reporters. Of the 32 original 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.

High Numbers of Confirmed Positive Clones

Due to the strong selective power of AbA resistance, first-round, low-stringency Matchmaker Gold screens use X-alpha-Gal and AbA to select only for blue, AbA-resistant colonies. Generally, two-thirds of the resulting putative positive clones can be later confirmed in secondary, high-stringency screens which select for all four Y2HGold reporters (Figure 4). As a result, we now recommend low-stringency screening to generate a large pool of colonies, followed by high-stringency clone verification. This strategy leads to greater numbers of genuine positives and fewer false positives than traditional, auxotrophic-based screens.

Aureobasidin A Resistance is Highly Selective

To demonstrate the selective properties of AbA resistance, we titrated the antibiotic against three diploid Y2HGold clones, each of which expressed a different combination of bait and prey proteins. Figure 5 shows that AbA selection clearly differentiated a negative control strain that expressed a non-interacting protein pair, from two strains that expressed interacting protein pairs.

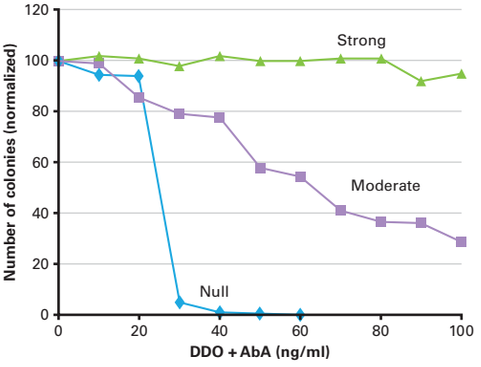

Figure 5. Aureobasidin A selection differentiates interacting bait and prey protein pairs. Y2HGold yeast clones expressing non-interacting (Null); moderately-interacting (Moderate); or strongly-interacting (Strong) protein pairs, were grown on DDO medium in the presence of increasing concentrations of AbA. Interacting protein pairs allowed yeast to grow at AbA concentrations much higher than 40 ng/ml.

Product	Size	Cat. No.
Matchmaker Gold Yeast Two-Hybrid System	each	630489
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	630462
	250 mg	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

Notice to Purchaser
For all licensing information, visit www.clontech.com

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. Complex Y2H library screens and your search for new protein interactions can now be accomplished in less time and with greater confidence than ever before.

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.

Matchmaker™ Gold 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) antibiotic 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 1), transcription of the Aureobasidin A resistance gene (AbA^r) is activated, allowing the cell to grow on medium containing 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.

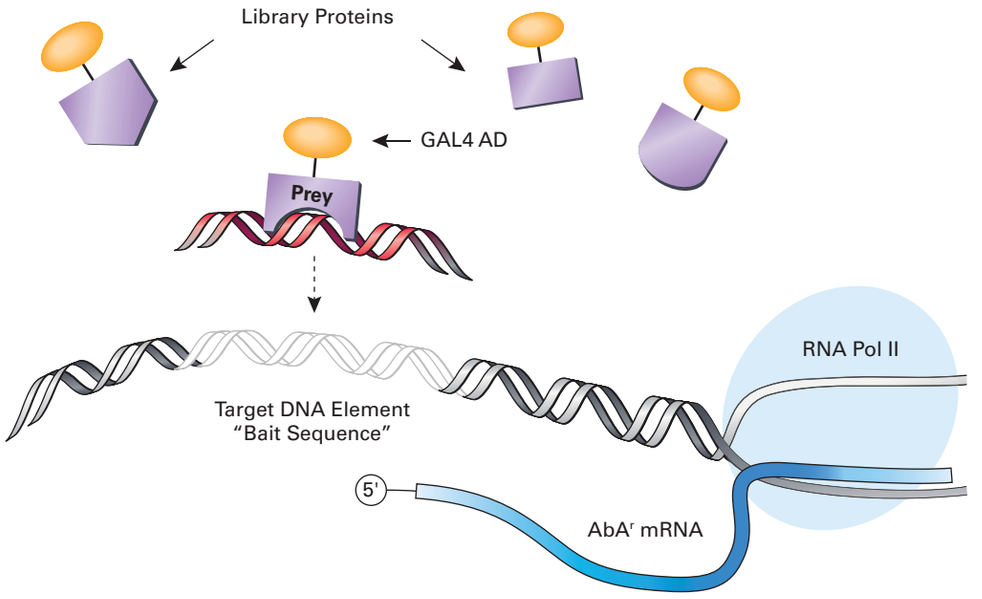

Figure 1. Screening for protein-DNA interactions with the Matchmaker Gold Yeast One-Hybrid System. Target-binding prey proteins from your cDNA library activate resistance to Aureobasidin A, which greatly simplifies the identification of positive clones.

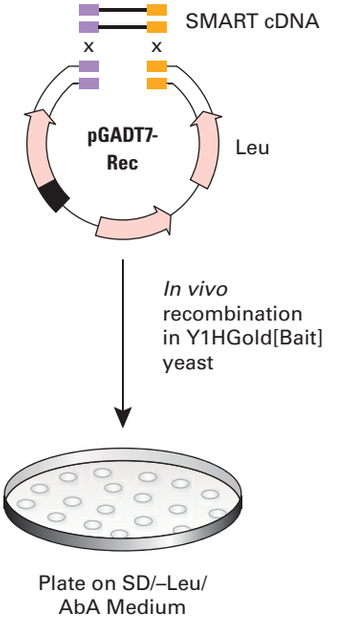

Figure 2. Use SMART technology and yeast biology to construct and screen your library. Your library is simultaneously constructed and screened directly in yeast. First, SMART cDNA synthesis technology is used to create a pool of cDNA that is flanked by sequences homologous to the ends of the linearized 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).

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

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,** Table I).

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 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. Pick the resulting colonies, which express 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.

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

Components

- pAbAi Vector
- pGADT7-Rec AD Cloning Vector
- SMART™ cDNA synthesis components
- Yeastmaker™ Yeast Transformation System 2
- Selection of Clontech Yeast Media Pouches

Notice to Purchaser

Please see the Advantage® and TITANIUM PCR Products, HotStart Antibody, PCR, SMART™ Amplification Products, and Aureobasidin Resistance Gene licensing statements on page 17.

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 2).

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.

Studying Interactions Involving 3 Proteins

Investigate enhancers and inhibitors of protein-protein interactions

- **pBridge expresses a third protein for performing modified two-hybrid studies**
- **"Bridge" proteins can positively or negatively affect two-hybrid interactions**

The yeast two-hybrid system (Y2H) has proven to be a powerful molecular tool for detecting protein-protein (binary) interactions involved in many molecular pathways. However, have you ever sought a third, or ternary, protein that disrupts or enhances such an interaction?

The **pBridge Vector** (Figure 1) is designed to express a "bridge" or third protein that participates in, or influences, yeast two-hybrid protein interactions; allowing higher order protein interactions to be investigated in a three-hybrid system (Figure 2).

Bridge Proteins Act:

- As a physical "bridge" that connects two proteins that do not interact with each other directly, or that stabilizes a weak interaction;
- As an inhibitor or modifier of one of the interacting proteins (e.g. a kinase).
- Alternatively, as a competitor of a two-hybrid interaction that is used to confirm the specificity of the interaction.

The conditional expression of the third protein allows investigation of its role in the interaction between bait and prey proteins in a Matchmaker System. Like the two-hybrid system, the three-hybrid system can be used to screen libraries to identify new interacting partners, with either the bridge protein or the bait protein as the target.

How Does It Work?

In order to study ternary interactions, use pBridge instead of pGBKT7 in the **Matchmaker™ Gold Yeast Two-Hybrid System.** Expression of the third protein is controlled by a conditional methionine-repressible promoter (P_{Met25}), i.e. it is expressed when methionine is absent from the growth medium.

In a Matchmaker Gold Yeast Two-Hybrid screen, positive interactions that activate the **Aureobasidin A** resistance reporter gene are detected by plating on selective media containing 70 ng/ml Aureobasidin A (AbA).

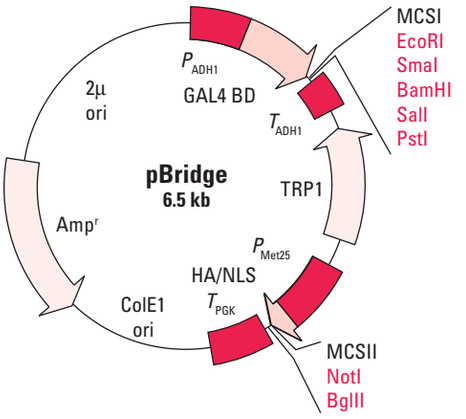

Figure 1. The pBridge Vector. The main target protein is cloned in MCSI and expressed as a fusion to the GAL4 DNA-BD, while a second "bridge" protein is cloned in MCSII and conditionally expressed from the P_{Met25} promoter.

Suggested Study Protocols

Three-hybrid screen to determine if the 3rd protein acts as a bridge or enhancer of bait-prey interactions:

1. Perform a two-hybrid screen using pBridge, but plate on SD/-Leu/-Met/-Trp/AbA media to express both bait proteins while selecting for AbA-resistance.
2. Next, patch-out the positive clones on selective, methionine-containing media that *represses* expression of the bridge protein (e.g. SD/-Leu/-Trp/AbA).
3. Colonies that do not survive the second plating contain candidate prey proteins that may be part of a productive ternary interaction involving the bridge protein and the BD bait.

Three-hybrid screen to determine if the 3rd protein acts as an inhibitor of protein-protein interactions:

1. Perform a two-hybrid screen using pBridge, plate on SD/-Leu/-Trp/AbA media to repress expression of the potential inhibitor while selecting for AbA-resistance.
2. Then patch-out the positive clones on selective media that activates expression of the bridge protein (SD/-Leu/-Met/-Trp/AbA).
3. Colonies that do not survive the second plating contain candidate prey proteins that interact with the BD bait, but that may be blocked by the bridge protein.

Product	Size	Cat. No.
pBridge Vector	20 µg	630404
Yeast Media Set 2 Plus	each	630495
Minimal SD Agar Base	467 g	630412
-Leu/-Met/-Trp DO Supplement	10 g	630430
Aureobasidin A	1 mg	630466

Notice to Purchaser
Please see the Aureobasidin Drug and pBridge Vector licensing statement at **www.clontech.com/licensing**

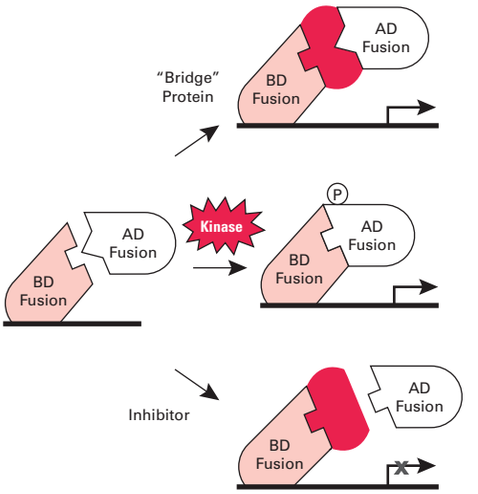

Figure 2. The Three-Hybrid System. Both the DNA-BD fusion and a candidate ternary-acting or "bridge" protein (red) are "bait" proteins and expressed from pBridge. The AD fusion "prey" protein is expressed from the pGADT7 vector in the Matchmaker Gold System. Bridge proteins can act as a physical bridge, modify one or both interacting proteins, or block a known interaction.

Matchmaker™ Mammalian Two-Hybrid System

Confirm protein-protein interactions in mammalian cells using a sensitive chemiluminescent assay

- **Test bait and prey proteins for their ability to interact in mammalian cells**
- **Simply cotransfect the cells, and sample the medium**
- **Allows hybrid proteins to attain native conformations and post-translational modifications**

The **Matchmaker Mammalian Assay Kit 2** is a two-hybrid system that allows you to test suspected protein-protein interactions in a mammalian cell background. Notably, the system can be used to validate novel binding relationships discovered in yeast two-hybrid screens.

Study and Validate Authentic Interactions

Performing two-hybrid screens in yeast is a powerful method of identifying novel protein binding relationships that involve a particular protein of interest. However, the conformation of a mammalian protein expressed in yeast may be quite different from its normal conformation in a mammalian cells. Thus, it is important to perform a two-hybrid assay in mammalian cells to confirm that the suspected interactions also take place when the proteins are folded and modified as they would be in their native environment. The mammalian assay often reflects interactions between mammalian proteins with greater authenticity than can be achieved in yeast.

Cotransfect and Assay for SEAP

To implement the system for a given pair of interacting proteins, one protein is fused to the GAL4 DNA-binding domain (DNA-BD) to create a bait protein, while the second is fused to the VP16 activation domain of HSV to create a prey protein (Figure 1). The two expression vectors are cotransfected into cells and if the bait and prey proteins interact, the two form a functional complex at the $P_{GAL4\text{-}E1b}$ promoter on the pG5SEAP reporter plasmid. This activates transcription of secreted alkaline phosphatase (SEAP), which is readily detected in the culture medium.

Characterize Interactions by Modifying the Proteins and Quantifying the Results

To further validate and characterize the interaction, the orientation of the proteins of interest can be reversed by swapping the bait domain for the prey, and vice versa. In addition, the coding regions can be modified or truncated to identify the domains or residues required for the interaction. The extent to which each modification affects the interaction can be easily quantified by measuring the SEAP activity produced by each combination and by then comparing their levels.

Assay SEAP Activity with a Convenient Kit

SEAP is an accurate and versatile reporter. It requires no cell lysis and is easily measured by sampling the culture medium and using the simple and highly sensitive assay provided by the **Great EscAPe SEAP Chemiluminescence Kit 2.0** (Figure 2). The kit is a complete system for detecting SEAP activity using the provided chemiluminescent substrate. SEAP activity is stable and can be left to accumulate over time so that even weak interactions can be assayed.

Product	Size	Cat. No.
Matchmaker Mammalian Assay Kit 2	each	630305
Great EscAPe SEAP Chemiluminescence Kit 2.0	50 rxns	631736
	300 rxns	631737
	1000 rxns	631738

Matchmaker™ Mammalian Assay Kit 2 Components

- pM GAL4 DNA-BD Cloning Vector
- pVP16 AD Cloning Vector
- pGG5SEAP Reporter Vector
- pM3-VP16 Positive Control Vector
- pM-53 Positive Control Vector
- pVP16-T Positive Control Vector
- pVP16-CP Negative Control Vector

Great EscAPe SEAP Chemiluminescence Kit 2.0 Components

- SEAP Substrate Solution
- 5X Dilution Buffer
- Positive Control Placental Alkaline Phosphatase

Notice to Purchaser

Please see the Matchmaker™ Two-Hybrid System licensing statements on page 17.

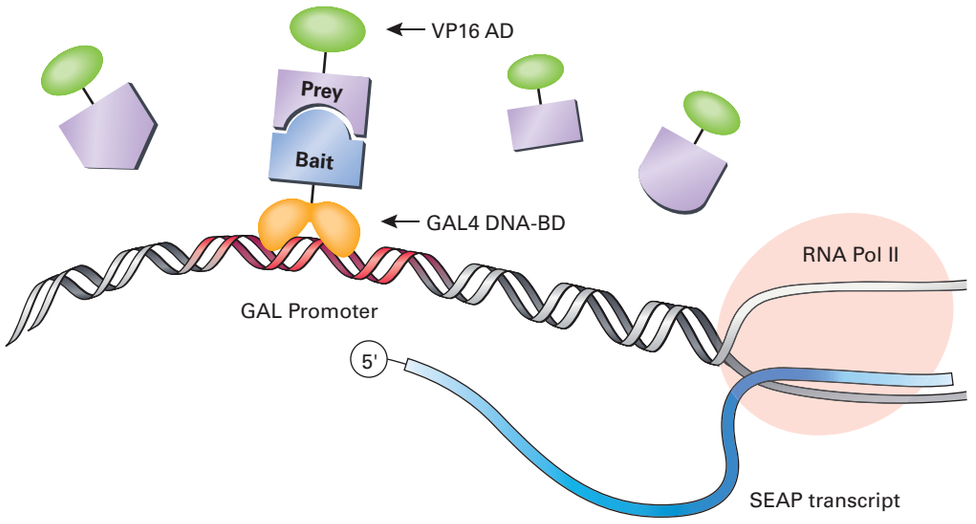

Figure 1. The mammalian two-hybrid principle. The bait protein is fused to the DNA binding domain of yeast GAL4 and the prey protein is fused to the transcriptional activation domain of HSV VP16. If the two proteins interact at the $P_{GAL4\text{-}E1b}$ promoter (GAL promoter), SEAP is secreted into the growth medium.

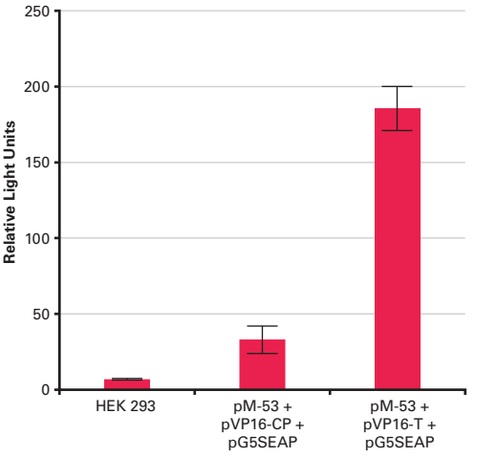

Figure 2. Chemiluminescent detection of protein-protein interactions. The bait vector pM-53 expresses p53 fused to the GAL4 DNA-binding domain. When HEK 293 cells were cotransfected with this bait, together with the SV40 large T antigen prey vector, pVP16-T, high levels of SEAP expression were detected due to the strong interaction between largeT antigen and p53.

Mate & Plate™ Yeast Two-Hybrid cDNA Libraries

Ready-made or make your own; by far the easiest screening method for yeast two-hybrid interactions

- **No library-scale plasmid amplification or transformation required for premade Mate & Plate Libraries**
- **Select from a wide variety of cDNA libraries: human, mouse, normalized & universal**
- **Make your own pretransformed library, simply and efficiently, using SMART™ technology**
- **Perfect for use with Matchmaker™ Gold Yeast Two-Hybrid Systems**

Clontech's Matchmaker Systems, including our latest version, the **Matchmaker Gold Yeast Two-Hybrid System** are highly optimized tools for screening cDNA libraries in yeast to identify novel DNA-binding proteins or protein-protein interactions (PPIs). Traditional construction of a GAL4-AD fusion protein library for yeast two-hybrid (Y2H) screening requires a significant amount of effort, including: cDNA synthesis, cloning the cDNA library in a yeast expression vector, library-scale amplification in *E. coli*, plasmid purification, and library-scale transformation into yeast—all this before screening can even begin.

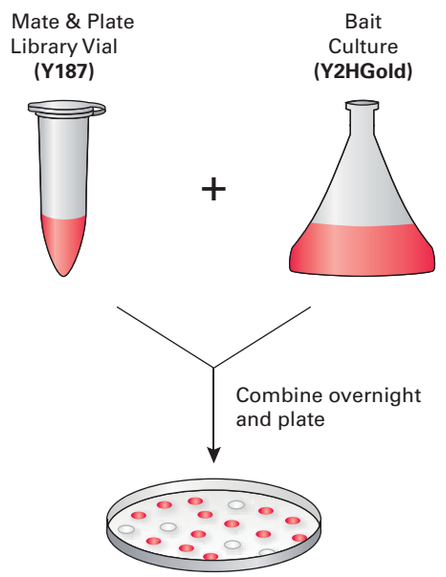

Figure 1. The Mate & Plate Protocol. To screen a Mate & Plate Library, an aliquot of the library in the *MATα* Y187 strain is simply mixed with a bait-expressing, *MATa* reporter strain culture (Y2HGold or AH109). The two strains are co-cultured overnight and then plated on selective agar medium.

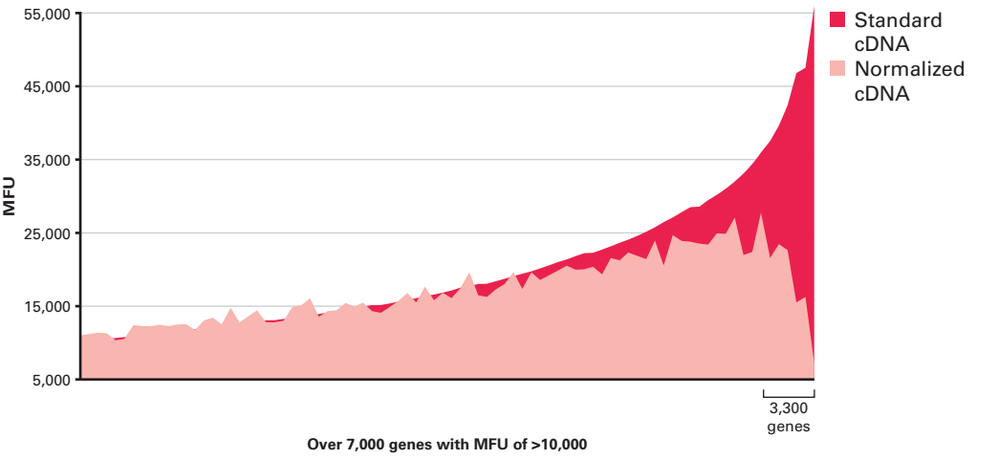

Figure 2. Normalization reduces the abundance of cDNAs derived from highly expressed genes. Universal cDNA, synthesized using RNA from mixed human tissues, was analyzed before and after normalization on a NimbleGen® *Homo sapiens* microarray (Cat. No. A4542-00-01). Data are shown for the 7,000+ genes which exhibited greater than 10,000 Mean Fluorescence Units (MFU). The signal intensities for approximately 3,300 of the most highly represented genes were significantly reduced following normalization, reflecting a preferential reduction of these abundant cDNAs.

Simple Screening with Mate & Plate Libraries

Fortunately, Clontech has an extensive variety of pretransformed cDNA libraries in yeast for which all the exhaustive steps of library construction and transformation have already been performed and verified. **Mate & Plate Libraries** are cDNA libraries of GAL4-AD prey fusion proteins that are ready for immediate screening in Y2H systems. These libraries have been transformed into the *MATα* haploid yeast strain, **Y187,** which can be easily mated to a haploid *MATa* reporter strain, such as **AH109** or **Y2HGold.**

Our "Mate & Plate" protocol makes library screening a very simple task accomplished by combining the Mate & Plate library culture with a culture of your bait-expressing reporter strain (Figure 1). Co-culturing the two strains overnight produces an array of diploid yeast clones, each coexpressing your bait with a different library prey protein. The clone pool can then be plated on selective media to screen for individual clones that express the appropriate reporter genes and markers, indicating the presence of interacting hybrid protein pairs.

Normalized Libraries Produce Fewer False Positives

Clontech also offers a selection of *normalized* Mate & Plate libraries, which further simplify the search for novel protein-protein interactions. Duplex-specific nuclease (DSN) normalization selectively removes abundant, and therefore redundant, cDNAs from the total pool and enriches the library for rare and less abundant sequences (1, 2; Figure 2). This process eliminates a major source of potential false positives. Library complexity effectively increases, which reduces the number of independent clones that must be screened in order to detect genuine positive interactions, and lowers the frequency of false positives that emerge from primary, low stringency screens.

By using a normalized Mate & Plate library together with the stringent screening methodology provided by the Matchmaker Gold System, your primary screens will greatly favor the identification of genuine positives, produce few false positives, and yield virtually no background colonies.

Mate & Plate™ Yeast Two-Hybrid cDNA Libraries...continued

Balanced Gene Representation

To illustrate how DSN normalization results in more balanced gene representation, cDNA samples taken before and after normalization were compared on a NimbleGen microarray containing 47,633 human genes (Figure 2). In this analysis, cDNA species from the most highly expressed genes were preferentially eliminated, while less abundant cDNAs remained largely unaffected. Consequently, the representation of low-copy-number sequences increased within the total cDNA pool. Figure 3 provides specific examples of how the cDNA levels of two highly-expressed housekeeping genes, β-actin and GAPDH, were effectively reduced by normalization.

Universal Libraries for Universal Gene Coverage

Our universal libraries provide the broadest and most complete coverage of genes that are expressed in almost any tissue. To create these all-purpose, normalized libraries, we combined RNAs from a diverse collection of either mouse or human whole tissues specifically chosen to represent an expansive range of expressed genes (3). These same RNA pools are used for our **qPCR Human Reference cDNA** and **Total RNA,** as well as for our **Human** and **Mouse Universal Reference Total RNAs** (3). Following cDNA synthesis and amplification using SMART™ technology, we normalize each cDNA pool before constructing and transforming the library into yeast. Combining "across-the-board" gene representation with the enrichment of low-copy-number cDNA, our universal normalized libraries offer the greatest capacity for identifying genuine binding partners of your protein of interest.

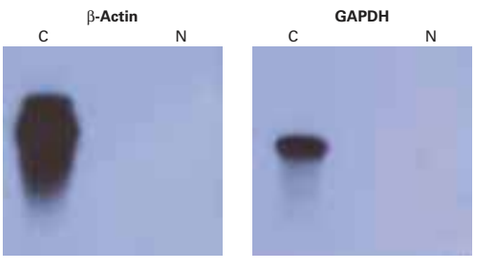

Figure 3. DSN-Normalization removes highly abundant cDNAs. Normalized (Lanes N) and non-normalized (Lanes C) human HeLa S3 cDNAs were compared using virtual Northern blot analysis and ^{32}P-labeled probes. The levels of these two highly abundant cDNAs were sharply reduced following normalization.

Efficient Two-Hybrid Screening

To illustrate the improved Y2H screening qualities of a normalized library, we searched for binding partners of a murine p53-bait, using our **Mate & Plate Library - HeLa S3 (Normalized).** A medium stringency screen of only 279,000 clones yielded 62 colonies that could possibly contain p53-binding proteins (1). In contrast, we recommend screening 1–2 million clones for a standard library. Of eight colonies that were selected for further analysis, four contained three different well-known binding partners of p53: PCNA, PRMT3, and PTEN. Thus, it is possible to screen a significantly smaller number of clones in a normalized library and still generate valuable data.

Make Your Own "Mate & Plate" Library

For researchers wishing to construct and transform their own cDNA library, we offer the convenient and straightforward **Make Your Own "Mate & Plate" Library System.** This system combines SMART technology with highly efficient homologous recombination in yeast, allowing you to efficiently synthesize cDNA and then clone it into the **pGADT7-Rec AD Cloning Vector** directly in yeast. Your library is constructed and transformed into the Y187 yeast strain in a single step. The result is a custom-made library ready to be used in our easy Mate & Plate protocol.

Mate & Plate Libraries, coupled with the stringent screening of the Matchmaker Gold, provide the most complete and advanced system for discovering new protein relationships. These tools afford you the greatest opportunities for Y2H screening success—and require the least amount of time and effort.

References

1. Pretransformed Normalized Matchmaker™ Library (January 2007) *Clontechniques* **XXII**(1):21–23.
2. Pretransformed Normalized Matchmaker™ Libraries (January 2008) *Clontechniques* **XXIII**(1):14–16.
3. High-Performance Reference RNA and cDNA (July 2008) *Clontechniques* **XXIII**(2):18–20.

Product	Size	Cat. No.
Mate & Plate Library - Universal Human (Normalized)		
	2 x 1 ml	630481
	5 x 1 ml	630480
Mate & Plate Library - Universal Mouse (Normalized)		
	2 x 1 ml	630482
	5 x 1 ml	630483
Mate & Plate Library - HeLa S3 (Normalized)		
	5 x 1 ml	630479
Mate & Plate Library - Human Bone Marrow		
	5 x 1 ml	630477
Mate & Plate Library - Human Fetal Brain		
	5 x 1 ml	630469
Mate & Plate Library - Human Heart		
	5 x 1 ml	630471
Mate & Plate Library - Human Liver		
	5 x 1 ml	630468
Mate & Plate Library - Human Skeletal Muscle		
	5 x 1 ml	630473
Mate & Plate Library - Human Testis		
	5 x 1 ml	630470
Mate & Plate Library - Human Ovary		
	5 x 1 ml	630474
Mate & Plate Library - Mouse Embryo 11-day		
	5 x 1 ml	630478
Mate & Plate Library - Mouse Embryo 17-day		
	5 x 1 ml	630476
Systems		
Make Your Own "Mate & Plate" Library System		
	5 rxns	630490
Matchmaker Gold Yeast Two-Hybrid System		
	each	630489

Notice to Purchaser

Please see the Aureobasidin Drug, Aureobasidin Drug Resistance Gene, Matchmaker™ Two-Hybrid System and Reverse Two-Hybrid System Technology , and SMART™ Amplification Products licensing statements on page 17.

Make Your Own "Mate & Plate™" Library for Yeast Two-Hybrid Screening

- **Library construction directly in yeast using SMART™ technology**
- **No laborious cloning or library amplification steps**
- **Enough material for hundreds of yeast two-hybrid screens**

Yeast two-hybrid systems are primarily used for screening a complete library of proteins (prey) for interaction with a specific protein of interest (bait). Traditional library manufacture and screening has always been time-consuming and labor-intensive. Not anymore—Clontech has developed a set of popular, ready-to-go libraries, called **Mate & Plate Libraries,** that require simple co-culturing of a library strain with your bait strain followed by plating on appropriate selective minimal medium. (1).

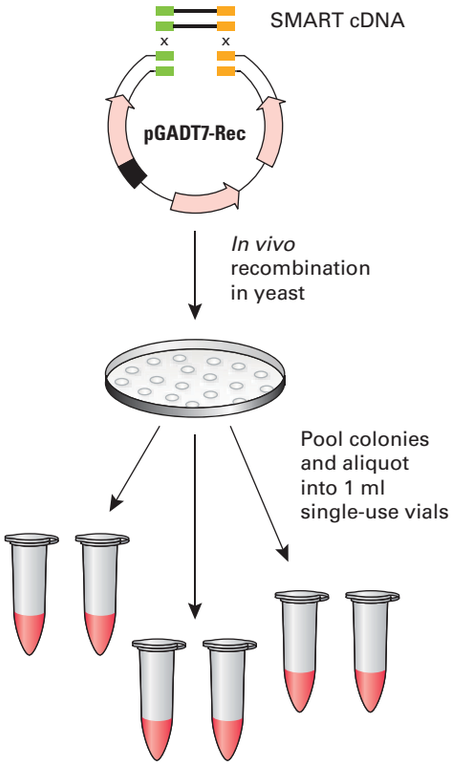

Figure 1. Library generation using *in vivo* recombination in *S. cerevisiae*. Mate & Plate Libraries are created via recombination between your cDNA and the Matchmaker prey vector pGADT7-Rec. The homologous sequences are generated by SMART cDNA synthesis. Colonies are pooled, mixed, and aliquoted into multiple vials. Each vial can be used for a two-hybrid screen.

Do-It-Yourself in Less than 7 Days

If the selection of ready-made libraries does not suit your needs, you can make your own library exactly the way we do, using our **Make Your Own "Mate & Plate" Library System.** Our kit provides the materials and methods to create enough library vials for hundreds of yeast two-hybrid screens—in less than a week.

Library creation occurs directly in our library **Y187 Yeast Strain,** utilizing the highly efficient homologous recombination machinery of *S. cerevisiae* (Figure 1). There is no need for the labor-intensive library cloning, amplification, and harvesting in *E. coli* that traditional library construction methods require. The system uses SMART cDNA synthesis technology, which allows you to construct cDNA libraries from any tissue source starting with as little as 100 ng of total RNA.

What is SMART Technology?

Clontech's SMART technology is based on two specific features of Moloney murine leukemia virus reverse transcriptase (MMLV RT):

- *Terminal transferase activity*
- *Template switching activity*

First-strand cDNA synthesis is primed by a modified oligo(dT) or random primer (Figure 2). When the SMART MMLV RT reaches the 5' end of the mRNA, the enzyme's *terminal transferase activity* attaches additional nucleotides, primarily dCTP, onto the newly synthesized strand of cDNA. Then the chemically modified SMART oligo, which contains a stretch of G residues at its 3' end, pairs with the extended dC-rich tail, serving as a second template onto which the RT enzyme *switches* to complete first-strand synthesis.

In the end, SMART cDNA synthesis incorporates known universal primer sequences at both ends of the cDNA. As a result, first-strand cDNA is:

- *Available for amplification by PCR.* You can start with nanogram amounts of RNA, allowing you to construct a library from microdissected tissues, laser-captured cells, biopsy samples, etc.
- *Homologous to the ends of the Matchmaker Gold prey plasmid, pGADT7-Rec.* The library is created by cotransformation of the Y187 Yeast Strain with pGADT7-Rec and SMART cDNA.

Let Yeast Do the Cloning

The highly efficient homologous recombination pathways of *S. cerevisiae* yeast have been well-documented (2, 3). Gap-repair has been exploited for decades by yeast biologists for the purpose of cloning plasmids in yeast (4). Clontech has taken this one step further by enabling *E. coli*-free cloning of entire libraries directly in yeast using homologous recombination. The entire process consists of just four steps:

Step 1. First-strand synthesis using SMART oligo to generate cDNA with known sequences at both ends that are homologous to pGADT7-Rec.

Step 2. Second-strand synthesis using PCR to generate 2–5 µg of library cDNA.

Step 3. Cotransformation and recombination of the cDNA and vector in the Y187 Yeast Strain.

Step 4. Harvest colonies, mix, and aliquot into 1 ml single use vials.

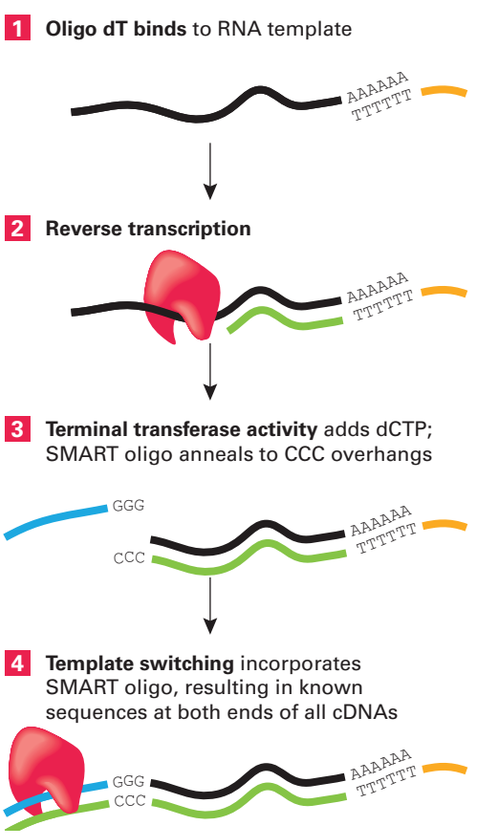

Figure 2. SMART cDNA synthesis generates cDNA ends with homology to pGADT7-Rec.

Make Your Own "Mate & Plate™" Library for Yeast Two-Hybrid Screening...continued

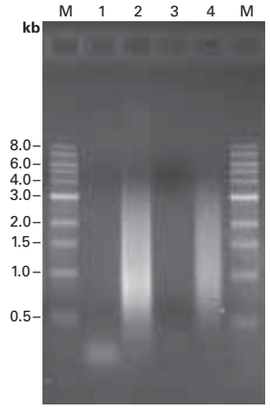

Figure 3. High-quality cDNA generated using SMART cDNA synthesis. Oligo dT-primed cDNA was generated using the Make Your Own "Mate & Plate" Library System. cDNA synthesis was carried out with or without 1 µg of human placenta polyA RNA (positive and negative controls, respectively). LD PCR was performed using the **Advantage® 2 Polymerase Mix** (with duplicate samples) and one set of products was purified (size-selected) using CHROMA SPIN™ TE 400 columns. Analysis of 10 µl of each sample on a 1% agarose gel revealed that the resulting cDNA ranged from 300 bp to 6 kb. Lanes M: 1 kb DNA ladder size marker. Lane 1: unpurified negative control. Lane 2: unpurified positive control. Lane 3: purified negative control. Lane 4: purified positive control. Lane 4 shows reduced abundance of cDNA below 400 bp compared to Lane 2, after size selection with CHROMA SPIN TE 400 columns.

Complex, Representative Libraries are Enriched for Longer cDNA Clones

The cDNA size range for inserts cloned using this protocol is 0.3–6 kb (Figure 3). Prematurely terminated reverse transcripts are selected against because they cannot be amplified using the second-strand synthesis primers, nor can they be cloned. This is because the terminally transferred SMART oligo sequence that is required for homology to the prey vector is not added to the cDNA if the RT falls from its template prematurely.

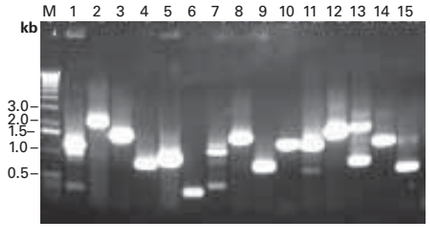

Figure 4. Mate & Plate libraries display broad insert representation. A human bone marrow library was made using the Make Your Own "Mate & Plate" Library System. Inserts from 15 randomly picked colonies were analyzed by yeast colony PCR using the Advantage 2 Polymerase Mix (Cat. No. 639201), and the Matchmaker AD LD-Insert Screening Amplimer Set (Cat. No. 630433). As seen in Lanes 1–15, every colony contained an insert of a different size. Lane M: 1 kb DNA ladder size marker.

The Make Your Own "Mate & Plate" Library System also contains CHROMA SPIN gel filtration columns to size-select for cDNAs larger than 400 bp. Figure 4 shows insert screening analysis of a Mate & Plate library made using human bone marrow RNA. Inserts were amplified from 15 yeast colonies chosen at random. Every clone contained an insert of a different size.

Since SMART technology has streamlined construction of complex cDNA libraries in yeast—you can Make Your Own "Mate & Plate" Library in less than 1 week!

References

1. Mate & Plate™ Yeast Two-Hybrid cDNA Libraries. (January 2009) *Clontechniques* **XXIV**(1):6–7.
2. Paques, F. and Haber, J. E. (1999) *Microbiol. Mol. Biol. Rev.* **63**(2):349–404.
3. Sung, P. *et al.* (2000) *Mutat. Res.* **451**(1–2):257–275.
4. Ma, H. (1987) *Gene* **58**(2–3):201–216.

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

Related Products

- Advantage® 2 Polymerase Mix and Kits (Cat. Nos. 639201, 639202, 639206 & 639207)

Notice to Purchaser

Please see the Aureobasidin Drug, Aureobasidin Resistance Gene, Matchmaker™ Two-Hybrid System, Reverse Two-Hybrid Technology, and SMART™ Amplification Products licensing statements on page 17.

High Quality Yeast Culture Media

Yeast media in convenient pouches and sets

Clontech provides yeast media in several convenient formats:

I. **Yeast Media Pouches** are ready-to-go preformulated pouches; just add water and autoclave. No measuring, mixing, or pH adjustments are required. Each pouch makes 0.5 L of media, with or without agar.

II. **Yeast Media Sets** are specifically designed for Clontech Matchmaker Gold Systems. The ready-to-go pouches are supplied in the quantities sufficient for the screening protocols described in the Matchmaker Gold User Manuals.

Yeast Media Pouches

Table I: Yeast Media Pouches

Yeast Media Pouches	Size	Cat. No.
Rich Media (for routine culturing of untransformed yeast)		
YPDA Broth	10 x 0.5 L	630306
YPDA with Agar	10 x 0.5 L	630307
Minimal Media Single Dropouts (SDO)		
SD/-Trp Broth	10 x 0.5 L	630308
SD/-Trp with Agar	10 x 0.5 L	630309
SD/-Leu Broth	10 x 0.5 L	630310
SD/-Leu with Agar	10 x 0.5 L	630311
SD/-His Broth	10 x 0.5 L	630312
SD/-His with Agar	10 x 0.5 L	630313
SD-Ura Broth	10 x 0.5 L	630314
SD/-Ura Broth with Agar	10 x 0.5 L	630315
Minimal Media Double Dropouts (DDO)		
SD/-Leu/-Trp Broth	10 x 0.5 L	630316
SD/-Leu/-Trp with Agar	10 x 0.5 L	630317
Minimal Media Triple Dropouts (TDO)		
SD/-His/-Leu/-Trp Broth	10 x 0.5 L	630318
SD/-His/-Leu/-Trp with Agar	10 x 0.5 L	630319
SD/-Leu/-Trp/-Ura Broth	10 x 0.5 L	630320
SD/-Leu/-Trp/-Ura with Agar	10 x 0.5 L	630321
Minimal Media Quadruple Dropouts (QDO)		
SD-Ade/-His/-Leu/-Trp Broth	10 x 0.5 L	630322
SD-Ade/-His/-Leu/-Trp with Agar	10 x 0.5 L	630323
SD/-His/-Leu/-Trp/-Ura Broth	10 x 0.5 L	630324
SD/-His/-Leu/-Trp/-Ura with Agar	10 x 0.5 L	630325

High Quality Yeast Culture Media...continued

Matchmaker™ Gold Yeast Media Sets

Clontech's Yeast Media Sets provide the perfect array of broth and agar growth media required for carrying out Matchmaker Gold protocols. The pouches included in each set are shown in Table II. Each pouch makes 0.5 L of media.

- **Yeast Media Sets 2** and **2 Plus** are for Matchmaker Gold Yeast Two-Hybrid protocols
- **Yeast Media Sets 1** and **1 Plus** are for Matchmaker Gold Yeast One-Hybrid protocols

Table II: Components of Matchmaker Gold Yeast Media Sets

	Yeast Media Set 2 (Cat. No. 630494)	**Yeast Media Set 1** (Cat. No. 630492)
Pouch Description	**Quantity**	**Quantity**
YPDA Broth	2	2
YPDA with Agar	1	1
SD/-Leu Broth	1	1
SD/-Leu with Agar	1	10
SD/-Ura with Agar	-	2
SD/-Trp Broth	1	-
SD/-Trp with Agar	1	-
SD/-Leu/-Trp with Agar	10	-
SD/-Ade/-His/-Leu/-Trp with Agar	1	-
Additional Components in Plus Sets	**Yeast Media Set 2 Plus** (Cat. No. 630495)	**Yeast Media Set 1 Plus** (Cat. No. 630493)
X-alpha-Gal	250 mg	-
Aureobasidin A	1 mg	1 mg

Table III: Media Supplements Available Separately

	Size	**Cat. No.**
X-alpha-Gal	100 mg	630462
X-alpha-Gal	250 mg	630463
Aureobasidin A	1 mg	630466

Notice to Purchaser
Please see the Aureobasidin Drug licensing statement on page 17.

Aureobasidin A: Selectable Drug Resistance for Yeast

A novel reporter gene, *AUR1-C,* confers resistance to the potent yeast antibiotic, Aureobasidin A

- **Aureobasidin A resistance is a definitive selectable marker for yeast**
- **Effectively reduces backgrounds in Matchmaker™ Gold yeast two-hybrid screens**
- **Identify genuine positives more easily, with fewer false positives**

Aureobasidin A (AbA) is a potent and unique yeast antibiotic that kills *S. cerevisiae* at low concentrations (1). The drug is a cyclic multipeptide (Figure 1) that acts by inhibiting an essential yeast enzyme, inositol phosphorylceramide synthase. A mutant enzyme, encoded by the *AUR1-C* gene, confers resistance to AbA and can be used as a very effective selectable marker that requires little or no optimization. The ability of resistant yeast to grow in the presence of AbA depends on the level of *AUR1-C* expression (Figure 5).

Aureobasidin A and Matchmaker Gold

Clontech's **Matchmaker Gold Yeast Two-Hybrid System**, is a highly optimized yeast two-hybrid (Y2H) screening system that employs the definitive selectivity of AbA resistance as a reporter for interacting Y2H protein pairs. The *AUR1-C* gene is stably integrated in the Matchmaker Gold reporter strain, **Y2HGold,** and is used for primary and secondary colony screens of Y2H libraries.

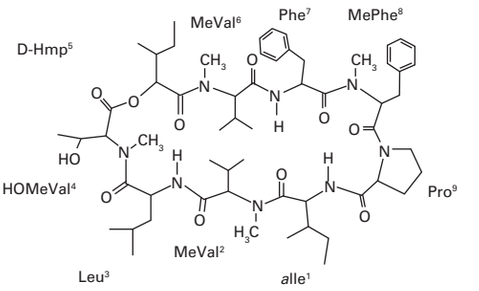

Figure 1. Structure of Aureobasidin A. Aureobasidin A (AbA; MW 1,100) is a cyclic depsipeptide antibiotic isolated from the fungus, *Aureobasidium pullulans* R106. AbA inhibits the product of the yeast AUR1 gene (inositol phosphorylceramide synthase) and is toxic to *S. cerevisiae* at low concentrations (0.1 µg/ml). The gene product of a dominant mutant allele, *AUR1-C,* confers resistance to AbA, and its expression can be used as a selectable marker.

Exceptionally Low Background

AbA selection virtually eliminates the high numbers of background colonies that often plague low stringency primary screens that use nutritional markers alone (e.g. *HIS3*). Because AbA actually kills sensitive cells, rather than merely retarding their growth, AbA-based selection greatly favors the growth and identification of genuinely positive clones. In general practice, a high percentage of clones that emerge from low stringency primary screens using AbA selection, are subsequently verified on high stringency secondary screens that select for all four Matchmaker Gold reporters (*AUR1-C, HIS3, ADE2* and *MEL1).*

Product	Size	Cat. No.
Aureobasidin A	1 mg	630466
Matchmaker Gold Yeast Two-Hybrid System	each	630489

Notice to Purchaser
Please see the Aureobasidin Drug, Aureobasidin Resistance Gene, Matchmaker™ Two-Hybrid System, and Reverse Two-Hybrid Technology licensing statements on page 17.

Like Ampicillin… for Yeast

Many researchers have yearned for a yeast selection system akin to those used for *E. coli* or mammalian cells. In fact, AbA is used for yeast essentially as ampicillin and kanamycin are used for cloning in *E. coli,* or as G418 is used to select stably transfected clones of mammalian cells. AbA resistance is far easier to use in Y2H library screening than are auxotrophic reporters, which often require optimization to achieve selective growth conditions.

If you wish to take your search for protein-protein interactions to the next level, look no further than Matchmaker Gold, and take advantage of the powerful and definitive selection of Aureobasidin A.

Reference

1. Takesako, K. *et. al* (1993) *J. Antibiot.* (Tokyo) **46**(9):1414–1420.

Transformation and Plasmid Isolation

Yeastmaker™ Yeast Transformation System 2

- **High-efficiency, small- and library-scale protocols**
- **YPD Plus medium elevates transformation efficiency up to 10^6 cfu/µg**
- **Optimized Yeastmaker Carrier DNA**

The **Yeastmaker™ Yeast Transformation System 2** provides a high-efficiency polyethylene glycol (PEG)/LiAc-based method for preparing and transforming competent yeast cells. The Yeastmaker protocol transforms yeast more efficiently and more reliably than many other commonly used methods. This is due to our highly optimized **YPD Plus Liquid Medium** and **Yeastmaker Carrier DNA.**

Product	Size	Cat. No.
Yeastmaker Yeast Transformation System 2	each	630439
Yeastmaker Carrier DNA	5 x 1 ml	630440

Applications

For transforming plasmids and other DNA into yeast.

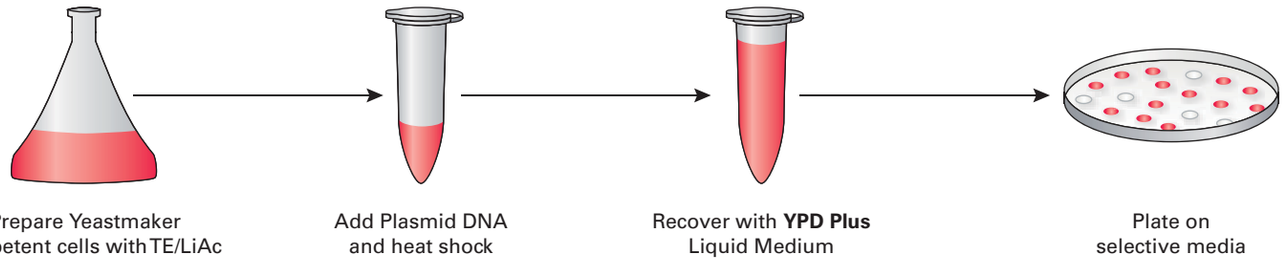

Figure 1. Optimized Yeastmaker Carrier DNA and YPD Plus recovery medium produce very high transformation efficiencies.

Easy Yeast Plasmid Isolation Kit

- **An easy, spin-column procedure**
- **Highly efficient cell wall digestion with Zymolyase enzyme**
- **Purify more DNA in less time**

The **Easy Yeast Plasmid Isolation Kit** provides a simple and efficient method for rescuing plasmid DNA from yeast *(Saccharomyces cerevisiae)*. The protocol uses Zymolyase to efficiently digest the cell walls of the yeast and generate spheroplasts, which are then subjected to SDS/alkaline lysis. A spin column purifies the plasmid DNA, which can then be transformed into *E. coli* for propagation and scaled-up plasmid preparations, or used as a template for PCR.

Product	Size	Cat. No.
Easy Yeast Plasmid Isolation Kit	50 preps	630467

Applications

For isolating plasmid DNA from transformed yeast clones, e.g. from yeast clones identified using the Matchmaker™ Systems.

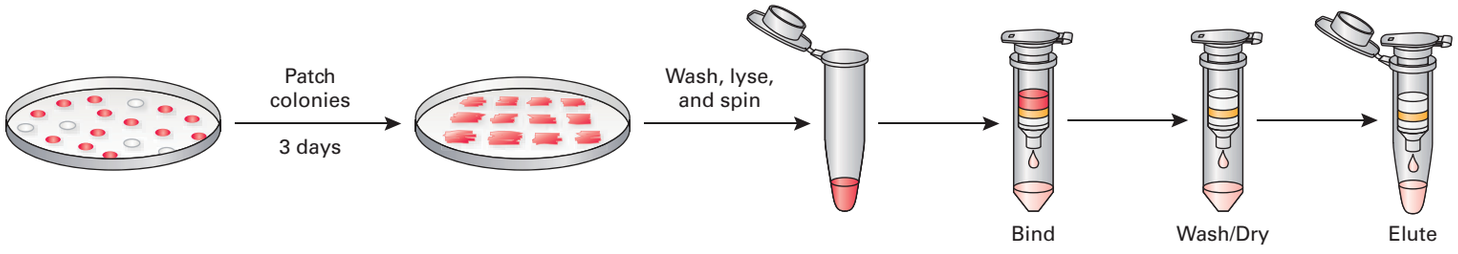

Figure 2. A simple digest, lyse, and spin column procedure for isolating plasmid DNA from yeast.

Rapidly Analyze the cDNA Inserts from Your Positive Clones

Characterize positive clones from one- and two-hybrid screens using colony PCR and a ready-made mix

- **Complete 2x mix for yeast colony PCR, including primers, dNTPs, and enzyme**
- **Sort clones and identify duplicates from library screens**
- **Works with a variety of Matchmaker library vectors**

Matchmaker Insert Check PCR Mix 2 is a ready-made premix for rapidly amplifying cDNA inserts in library vectors directly from yeast colonies. It's designed to be used with our **Matchmaker Gold** yeast one- and two-hybrid library screening systems, and allows you to quickly amplify, sort, and analyze the cDNA inserts in positive clones.

With this convenient mix, there is no need to isolate yeast plasmid DNA, transform *E. coli,* or make minipreps in order to characterize your library inserts. You simply pick a yeast colony whose phenotype has passed your screening criteria, disperse it into 25 µl of water, add the 2X mix, and amplify the mixture in a thermocycler (Figure 1). The amplified cDNA inserts are then ready for agarose gel analysis or for restriction enzyme digestion (Figure 2).

The Matchmaker Insert Check PCR Mix 2 is a complete mix that contains a PCR polymerase, primers, dNTPs and buffer; simply add the mix to yeast cells diluted in water, and perform 30 cycles of PCR.

The mix is compatible with GAL4 AD libraries constructed in the following vectors: pGADT7, pGADT7-Rec, pGADT7-Rec2, pGAD424, pACT, pACT2, pGAD GH, pGAD GL, and pGAD10.

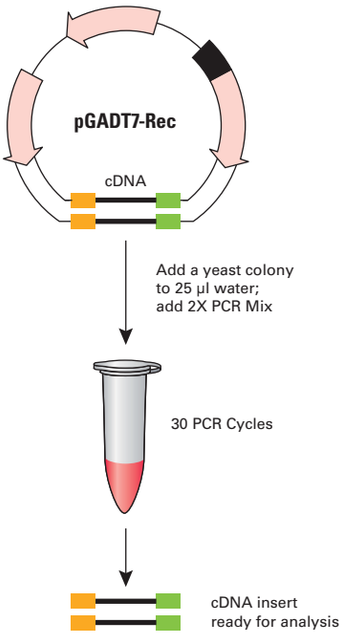

Figure 1. Colony PCR is quick and easy. With the Matchmaker Insert Check PCR Mix 2 protocol, transfer a colony into water, add the 2X mix, and amplify. The PCR reactions can be analyzed directly on an agarose gel or used for restriction fragment analysis.

Product	Size	Cat. No.
Matchmaker Insert Check PCR Mix 2	100 rxns	630497

Matchmaker™ Insert Check PCR Mix 2 Components

- 4 tubes Matchmaker Insert Check Mix 2 (625 µl each)

Notice to Purchaser

Please see the Advantage® and TITANIUM PCR Products, Hot Start Antibody, and PCR licensing statements on page 17.

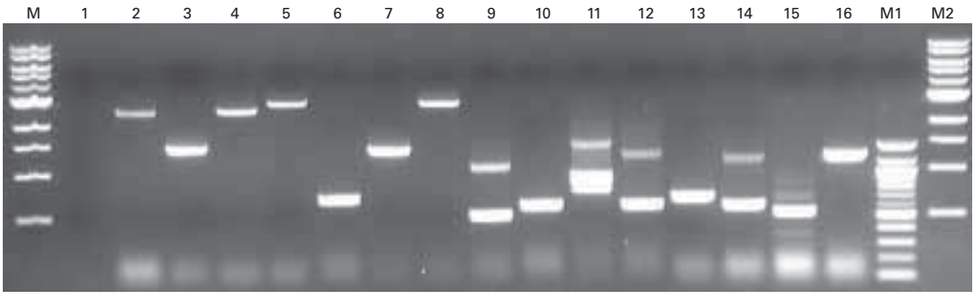

Figure 2. Quickly analyze and sort your positive candidate clones. Matchmaker Insert Check PCR Mix 2 was used to amplify prey vector inserts from 15 randomly selected colonies containing candidate clones obtained with our Matchmaker Gold System (Lanes 3-17). The results allowed the clones to be quickly sorted for further analysis. Lane 2: No template control. Lanes M1: 1 kb ladder. Lane M2: 100 bp ladder.

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NimbleGen® is a registered trademark of Roche NimbleGen, Inc.

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Licensing Statements

Advantage® and TITANIUM™ PCR Products

U.S. Patent No. 5,436,149 for LA Technology is owned by Takara Bio Inc.

Aureobasidin Drug

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Hot Start Antibody

TaqStart® Antibody and other Hot Start Antibodies are licensed under U.S. Patent No. 5,338,671.

Matchmaker™ Two-Hybrid System

Practice of the two-hybrid system is covered by U.S. Patents No. 5,283,173, No. 5,468,614, and No. 5,667,973 assigned to the Research Foundation of the State University of New York. Purchase of any Clontech two-hybrid reagent does not imply or convey a license to practice the two-hybrid system covered by these patents. Commercial entities purchasing these reagents must obtain a license from the Research Foundation of the State University of New York before using them. Clontech is required by its licensing agreement to submit a report of all purchasers of two-hybrid reagents to SUNY Stony Brook. Please contact SUNY Stony Brook for license information (Tel: 631-632-9009; Fax: 631-632-1505).

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PCR

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When reviewing any license statements herein, please be advised that the following patents have expired, and the license statements are hereby amended to that effect.

U.S. Patent Nos.

4,876,194	4,888,274	4,889,818	5,468,614	5,667,973	2,005,016
5,942,610	5,141,813	5,401,837	5,451,463	4,745,051	4,879,236
4,937,190	5,021,344	5,057,493	5,079,352	5,118,620	5,122,458
5,126,251	5,135,855	5,168,062	5,173,418	5,210,015	5,225,348
5,266,491	5,283,173	5,385,839	5,487,972	6,054,270	6,214,979
4,683,202	4,695,548	4,861,448	4,788,135	4,686,186	4,683,195
4,965,188	6,475,440	5,994,076	5,989,872	5,663,316	6,489,159

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