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, see pages 6–7 of this issue).

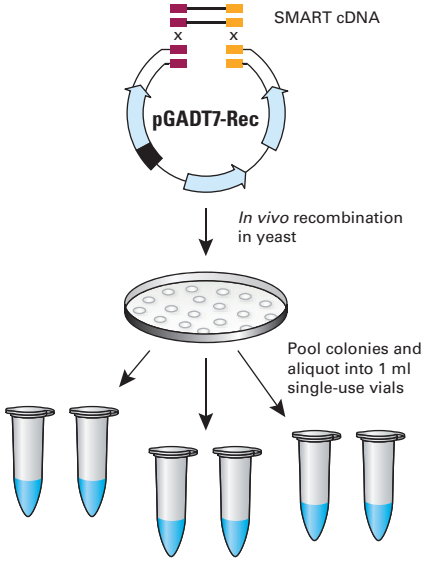

Figure 1. Library generation using *in vivo* recombination in *S. cerevisiae.* Mate & Plate Libraries are created via recombination between your cDNA and the Matchmaker prey vector pGADT7-Rec. The homologous sequences are generated by SMART cDNA synthesis. Colonies are pooled, mixed, and aliquoted into 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 just 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.

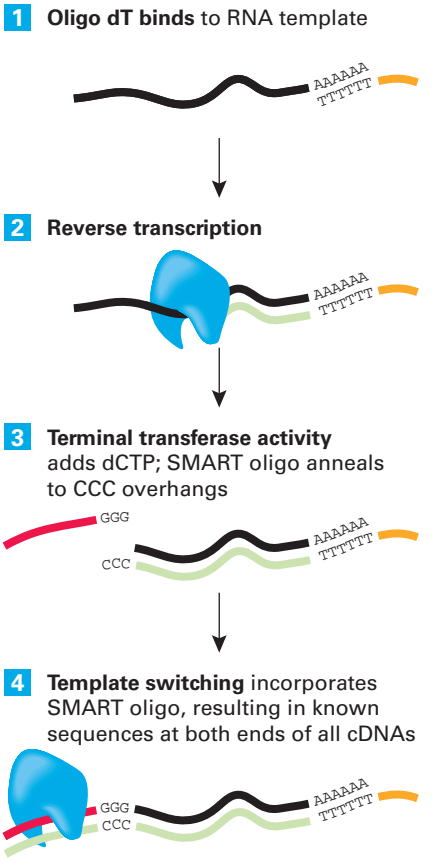

Figure 2. SMART cDNA synthesis generates cDNA ends with homology to pGADT7-Rec.

Make Your Own "Mate & Plate™" Library...continued

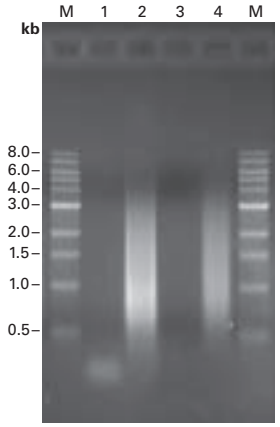

Figure 3. High-quality cDNA generated using SMART cDNA synthesis. Oligo dT-primed cDNA was generated using the Make Your Own "Mate & Plate" Library System. cDNA synthesis was carried out with or without 1 µg of human placenta polyA RNA (positive and negative controls, respectively). LD PCR was performed 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.

Let Yeast Do the Cloning

The highly efficient homologous recombination pathways of *S. cerevisiae* yeast have been well-documented (2, 3). Their efficiency has been exploited for decades by yeast biologists for the purpose of *E. coli*-free cloning of plasmids, a process known as Gap-repair (4). Clontech has taken this one step further by enabling *E. coli*-free cloning of an entire library 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 PCR synthesis to generate 2–5 µg of library cDNA.

Step 3. Cotransformation and recombination in the Y187 Yeast Strain.

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

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.

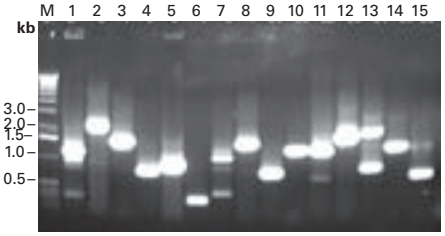

Figure 4. Mate & Plate libraries display broad insert representation. A human bone marrow library was made using the Make Your Own "Mate & Plate" Library System. Inserts from 15 randomly picked colonies were analyzed by yeast colony PCR using the Advantage 2 Polymerase Mix (Cat. No. 639201), and the 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.

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 Matchmaker™ Two-Hybrid System, Reverse Two-Hybrid Technology, and SMART™ Amplification Products licensing statements on page 40.

The Make Your Own "Mate & Plate" Library System also contains CHROMA SPIN gel filtration columns to size-select for larger cDNAs. Those larger than 400 bp are selected for as part of the protocol. 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.