Make Your Own "Mate & Plate™" Library System

**Catalog No.** 630490  **Amount** 5 rxns  **Lot Number** Specified on product label.

**Description**
Mate & Plate Libraries are by far the easiest libraries to screen for protein-protein interactions using a GAL4 yeast two-hybrid system. Several Mate & Plate Libraries are available ready-made from Takara Bio USA, Inc. For libraries that are not available, this system provides the necessary components and a simple, highly efficient method to make your own Mate & Plate Library using SMART® technology and the highly efficient homologous recombination machinery of *Saccharomyces cerevisiae*.

**Package Contents**

**Box 1:**
- 10 µl SMART MMLV RT (200 units/µl; also available as Cat. No. 639522)
- 300 µl 5X First-Strand Buffer
- 165 µl DTT (100 mM)
- 10 µl CDS III Primer (12 µM)
- 10 µl CDS III/6 Primer (10 µM)
- 7 µl RNase H (2 units/µl)
- 50 µl 5’ PCR Primer (10 µM)
- 50 µl 3’ PCR Primer (10 µM)
- 500 µl Melting Solution
- 20 µl SV40 Large T PCR Fragment (25 ng/µl)
- 50 µl dNTP Mix (10 mM each dNTP)
- 25 µg pGADT7-Rec AD Cloning Vector (Sma I-linearized; 500 ng/µl)

**Box 2:**
- Yeastmaker™ Yeast Transformation System 2 (Box 1 of 2):
  - 2 x 1 ml Yeastmaker Carrier DNA, denatured (10 mg/ml)
  - 20 µl pGBT9 (100 ng/µl; control plasmid)

**Box 3:**
- Yeastmaker Yeast Transformation System 2 (Box 2 of 2):
  - 2 x 50 ml 50% PEG
  - 50 ml 1 M LiAc (10X)
  - 50 ml 10X TE Buffer
  - 50 ml YPD Plus Liquid Medium

**Box 4:**
- 10 µl SMART III Oligonucleotide (12 µM)
- 5 µl Control Poly A+ RNA (Mouse Liver; 1 µg/µl)
- 0.5 ml *S. cerevisiae* Y187
Box 5:
- 1 pouch YPDA Broth (0.5 L)
- 1 pouch YPDA with Agar (0.5 L)
- 1 pouch SD/Leu with Agar (0.5 L)
- 50 ml NaCl Solution (0.9%)
- 300 µl Sodium Acetate (3 M; pH 4.8)
- 500 µl Deionized H₂O
- 10 each CHROMA SPINTM+TE-400 Columns

Storage Conditions
- Store Boxes 1 & 2 at −20°C.
- Store Box 4 at −70°C.
- Store Boxes 3 & 5 at room temperature.

Expiration Date
- Specified on product label.

Shipping Conditions
- Boxes 1, 2 & 4: Dry ice (−70°C)
- Boxes 3 & 5: Room temperature

Product Documents
Documents for our products are available for download at takarabio.com/manuals
The following documents apply to this product:

- Make Your Own "Mate & Plate" Library System User Manual
- pGADT7-Rec Vector Information

Quality Control Data

1. Plasmid Identity and Purity
   a. The identity of the pGADT7-Rec Vector was verified by agarose/EtBr gel electrophoresis after digestion with the indicated enzyme. The purity of the vector was checked by determining the A₂₆₀/A₂₈₀.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Enzyme(s)</th>
<th>Fragment Size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGADT7-Rec</td>
<td>SmaI</td>
<td>8.0</td>
</tr>
</tbody>
</table>

A₂₆₀/A₂₈₀: 1.8–2.0

b. The pGADT7-Rec AD Cloning Vector (SmaI-linearized) was also checked by transformation into Stellar™ Competent Cells (Cat. No. 636763). Transformants were selected by plating on LB/Amp (100 µg/ml). The linearized vector produced ≤ 0.5% of the number of colonies produced with circular (uncut) pGADT7-Rec.

2. Yeast Strain
   The nutritional requirements (i.e., auxotrophic markers) of the Y187 strain were verified by streaking samples onto several types of SD minimal media.
3. cDNA Synthesis & Recombination-mediated Cloning

a. Single-stranded cDNA was generated from 1 µg of Control Mouse Liver Poly A+ RNA using SMART MMLV Reverse Transcriptase as described in the User Manual (PT4085-1). Two samples of first-strand cDNA were prepared: One was generated with the CDS III Primer; the other, with the CDS III/6 Primer. Next, 2 µl of each cDNA sample was amplified by PCR, to prepare ds cDNA as described in the User Manual. Finally, 5 µl of the PCR product was electrophoresed on a 1.2% agarose/EtBr gel. A moderately strong smear from ≥0.1 kb to 4 kb (or more) was observed.

b. Recombination-mediated cloning was tested with the following four transformations:

<table>
<thead>
<tr>
<th>Transformation</th>
<th>Competent Y187 cells</th>
<th>ds cDNA</th>
<th>pGADT7-Rec2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100 µl</td>
<td>5 µl</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2.</td>
<td>100 µl</td>
<td>—</td>
<td>5 µl</td>
<td>—</td>
</tr>
<tr>
<td>3.</td>
<td>100 µl</td>
<td>—</td>
<td>5 µl</td>
<td>—</td>
</tr>
<tr>
<td>4.</td>
<td>100 µl</td>
<td>—</td>
<td>5 µl</td>
<td>—</td>
</tr>
</tbody>
</table>

*a First-strand cDNA was synthesized as in Part 3a, above, using either the CDS III or CDS III/6 Primer. The cDNA was then PCR amplified, purified by gel filtration (using a CHROMA SPIN+TE-400 Column, Cat. No. 636076), and finally, concentrated by ethanol precipitation, all as described in PT4085-1.

*b The SV40 Large T PCR Fragment provided with this kit.

*c The Blank was prepared and processed in the same way as the CDS III and CDS III/6 samples with one exception: Mouse Liver Poly A+ RNA was omitted from the first-strand synthesis reaction.

Following transformation, the mixtures were spread on SD/-Leu plates and incubated at 30°C.

Transformation #4, a negative control, produced ≤20% of the number of colonies observed for Transformations #1 or #2, and Transformation #3 produced ≥4X the number of colonies observed for Transformation #4.

4. Yeast Mating

Mating functionality was confirmed by performing the following two matings:


b. Y2HGold[pGBK7-lam] x Y187[pGADT7-T] (negative control mating)

Expected colony growth was observed on SD/-Leu/-Trp media and SD/-Leu/-Trp/-Ade/-His media containing Aureobasidin A and X-alpha-Gal.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.
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CATALOG NO.
630490

NOTICE TO PURCHASER:

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