Certificate of Analysis

Make Your Own "Mate & Plate™" Library System

Catalog No.	Amount	Lot Number
630490	5 rxns	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 H_2O
- 10 each CHROMA SPIN™+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
- Yeastmaker Yeast Transformation System 2 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 A_{260}/A_{280}.

Vector	Enzyme(s)	Fragment Size (kb)
pGADT7-Rec	SmaI	8.0

A_{260}/A_{280}: 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:

		ds cDNA				pGADT7-Rec2
Transformation	**Competent Y187 cells**	**CDS III[a]**	**CDS III/6[a]**	**SV40[b]**	**Blank[c]**	**(SmaI-linearized)**
1.	100 µl	5 µl	—	—	—	1 µl
2.	100 µl	—	5 µl	—	—	1 µl
3.	100 µl	—	—	5 µl	—	1 µl
4.	100 µl	—	—	—	5 µl	1 µl

[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 2 matings:

a. Y2HGold[pGBKT7-53] x Y187[pGADT7-T] (positive control mating)

b. Y2HGold[pGBKT7-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.

TaKaRa

Make Your Own "Mate & Plate™" Library System

CATALOG NO.

630490

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with the licensing requirements, listed below if applicable, and described on the product´s web page at http://www.takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

TRADEMARKS:

© 2010 Takara Bio Inc. All Rights Reserved.
All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Certain trademarks may not be registered in all jurisdictions.

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada	**Asia Pacific**	**Europe**	**Japan**
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999