Certificate of Analysis

Matchmaker® Gold Yeast One-Hybrid Library Screening System

Catalog No.	Amount	Lot Number
630491	5 rxns	Specified on product label.

Description

A simple and highly efficient system for the simultaneous construction and screening of a cDNA library for protein-DNA interactions. SMART® cDNA is synthesized from your RNA sample and then used to construct a library directly in a Y1HGold yeast reporter strain containing your DNA sequence of interest. Positive protein-DNA interactions from the library convey resistance to the yeast antibiotic, Aureobasidin A (AbA).

Package Contents

Package 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)
- 50 µl 5' PCR Primer (10 µM)
- 50 µl 3' PCR Primer (10 µM)
- 7 µl RNase H (2 units/µl)
- 500 µl Melting Solution
- 50 µl dNTP Mix (10 mM each dNTP)
- 25 µg pGADT7-Rec AD Cloning Vector (Sma I-linearized; 500 ng/µl)
- 20 µg pAbAi Vector (500 ng/µl)
- 20 µl p53-AbAi Control Vector (500 ng/µl)
- 25 µl p53 Control Insert (25 ng/µl)
- 50 µl pGADT7 AD Cloning Vector (100 ng/µl)

Package 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)

Package 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

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Package 4:

- 10 µl SMART III Oligonucleotide (12 µM)
- 5 µl Control Poly A^+ RNA (Mouse Liver; 1 µg/µl)
- 0.5 ml *S. cerevisiae* Y1HGold

Package 5:

- 1 pouch YPDA Broth (0.5 L)
- 1 pouch YPDA with Agar (0.5 L)
- 1 pouch SD/-Ura 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 Packages 1 & 2 at –20°C.
- Store Package 4 at –70°C.
- Store Packages 3 & 5 at room temperature.

Expiration Date

- Specified on product label.

Shipping Conditions

- Packages 1, 2 & 4: Dry ice
- Packages 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:

- Matchmaker Gold Yeast One-Hybrid Library Screening System User Manual
- Yeastmaker Yeast Transformation System 2 User Manual
- pGADT7-Rec Vector Information
- pGADT7-Rec Vector Sequence in GenBank Format
- pAbAi Vector Information
- pAbAi Vector Sequence in GenBank Format
- pGADT7 AD Vector Information
- pGADT7 AD Vector Sequence in GenBank Format

Quality Control Data

Plasmid Identity and Purity

- The identity of the included vectors was verified by agarose/EtBr gel electrophoresis after digestion with the indicated enzyme.

Vector	Enzymes	Fragments
pGADT7-Rec	SmaI	8.0 kb
pAbAi	BstBI	4.9 kb
	BbsI	4.9 kb
	HindIII/PvuII	3.0 & 1.8 kb
p53-AbAi	BstBI	4.9 kb
	BamHI	4.6 & 0.3 kb
pGADT7 AD	EcoRI	8.0 kb
	HindIII	7.2 & 0.8 kb

- A_{260}/A_{280}: 1.8–2.0 for each vector.
- The pGADT7-Rec AD Cloning Vector (SmaI-linearized) was also checked by transformation into Stellar™ Competent Cells (Takara Bio, 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.

Yeast Strain

The nutritional requirements (i.e., auxotrophic markers) of the Y1HGold strain were verified by streaking samples onto several types of SD minimal media.

cDNA Synthesis & Recombination-mediated Cloning

- 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 (PT4087-1). 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.

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

	Competent	ds cDNA				pGADT7-Rec
Transformation	Y1HGold[p53-AbAi] cells	CDS III*	CDS III/6*	P53†	Blank‡	(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

*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 PT4087-1.

†p53 Control Insert

‡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 agar medium and incubated at 30°C. Transformation #4, a negative control, produced ≤20% of the number of colonies observed for Transformations #1 or #2.

- Detection of the yeast one-hybrid interaction between p53 and the p53 cis-DNA consensus binding element

  Transformation #3, in Part 3B above, yielded transformants when plated on SD/-Leu agar + 200 ng/ml Aureobasidin A, while Y1HGold[p53-AbAi] transformed with the pGADT7-Rec Vector alone (circular) gave less than 2% of the number of colonies obtained for Transformation #3 on the SD/-Leu agar + 200 ng/ml Aureobasidin A.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

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CATALOG NO.

630491

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:

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