

Matchmaker® Gold Yeast One-Hybrid Library Screening System

Catalog No(s).

630491

Amount

5 rxns

Lot Number

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

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)
- 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)

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* Y1HGold

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Box 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 H₂O
- 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.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

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:

- Matchmaker Gold Yeast One-Hybrid Library Screening System User Manual (PT4087-1)
- Yeastmaker Yeast Transformation System 2 User Manual (PT1172-1)
- pGADT7-Rec Vector Information (PT3530-5)
- pAbAi Vector Information (PT4091-5)
- pGADT7 AD Vector Information (PT3249-5)

Quality Control Data

1. Plasmid Identity and Purity

- The identity of the included vectors 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.

Vector	Enzyme(s)	Fragment Size (kb)
pGADT7-Rec	SmaI	8.0
pAbAi	BstBI	4.9
	BbsI	4.9
	HindIII/PvuII	3.0, 1.8
p53-AbAi	BstBI	4.9
	BamHI	4.6, 0.3
pGADT7 AD	EcoRI	8.0
	HindIII	7.2, 0.8

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 $\leq 0.5\%$ of the number of colonies produced with circular (uncut) pGADT7-Rec.

2. Yeast Strain

The nutritional requirements (i.e., auxotrophic markers) of the Y1HGold 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 (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.
- b. Recombination-mediated cloning was tested with the following four transformations:

Transformation	Competent	ds cDNA				pGADT7-Rec (SmaI-linearized)
	Y1HGold[p53-AbAi] cells	CDS III ^a	CDS III/6 ^a	P53 ^b	Blank ^c	
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 PT4087-1.

^b p53 Control Insert

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

- c. 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.

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