Certificate of Analysis

Mate & Plate™ Library - HeLa S3 (Normalized)

Catalog No.	Lot Number
630479	1707551A

Description

This yeast two-hybrid library was constructed from HeLa S3 cDNA that had been previously normalized to preferentially remove abundant cDNAs derived from high-copy-number mRNAs. The normalization process incorporates a Duplex-Specific Nuclease (DSN) treatment and SMART® technology and increases the representation of low-copy-number transcripts in the library. This reduces the number of clones that must be screened to identify positive interactions and facilitates the identification and characterization of novel protein-protein interactions.

HeLa S3 cDNA library transformed into yeast strain Y187. The library can be readily mated to a *MAT**a** GAL4* reporter strain, such as AH109 or Y2HGold (Clontechniques, 2008).

Package Contents

- 5 x 1.0 ml Mate & Plate Library HeLa S3 (Normalized))
- 1 x 1.0 ml Mate & Plate Library - Control (pGADT7-T in Y187)

Storage Conditions

- Store all components at –70°C
- Do not refreeze

Shelf Life

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

mRNA Source

- Cervical Adenocarcinoma (HeLa S3; a clonal derivative of the parent HeLa cell line) poly A+ RNA. ATCC No. CCL2.2.

Cloning Vector

- pGADT7-RecAB

Cloning Site

- Sfi I A/Sfi I B

Priming Method

- Sfi I $(dT)_{30}$ primed

Yeast Genotype

- Y187: *MATa, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, met–, URA3 :: GAL1*$_{UAS}$*-GAL1*$_{TATA}$*-LacZ, MEL1*

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(102218)			

Shipping Conditions

- Dry ice (–70°C)

Product Documents

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

- Matchmaker® Gold Yeast Two-Hybrid System User Manual (PT4084-1)
- pGADT7-RecAB Vector Information (PT3718-5)

References

Pretransformed Mate & Plate Libraries (January 2008) Clontechniques **XXiV**(1):X-Y.
Zhulidov, P.A., et al. (2004) Nucleic Acids Res. **32**:e37.
Shagin, D.A., et al. (2002) Genom Res. **12**:1942–1953.
Franz, O., et al. (1999) Nucleic Acids Res. **27**:e3.

Quality Control Data

1. **Quality Control Data**

Test	Result
Titer (yeast colonies)	>5 x 10^7 cfu/ml
Number of independent clones	2.24 x 10^6
Average cDNA size	1.49 kb
cDNA size range*	0.9 – 2.7 kb

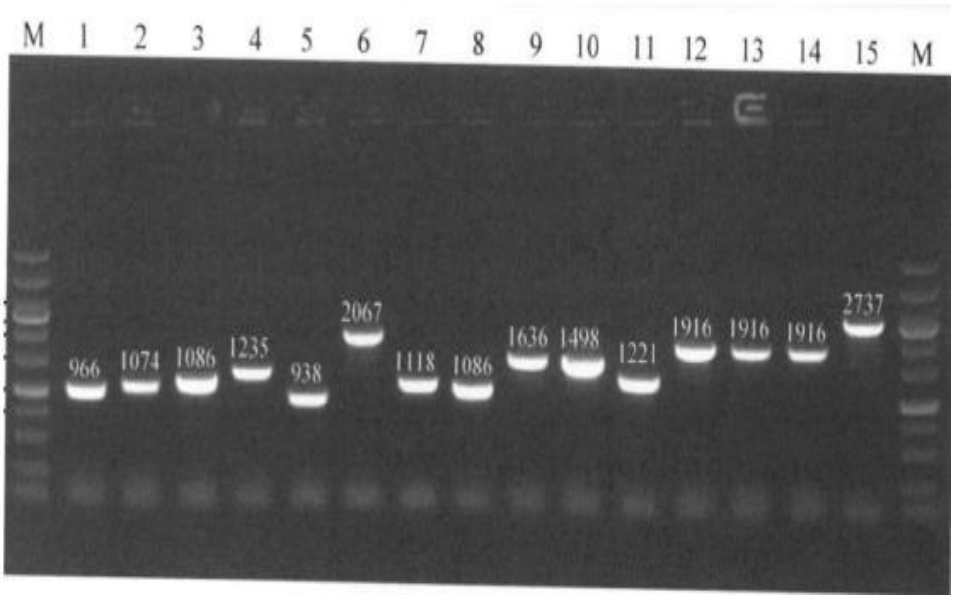
*the cDNA was size-selected by excision from an agarose gel prior to cloning

2. **Quality Control Data for the Pretransformed Library in Yeast**

Library Insert Size Screening
15 yeast colonies were randomly picked and screened by PCR using the Matchmaker AD LD-Insert Screening Amplimer Set (Cat. No. 630433).

15 of 15 colonies contained inserts as determined by PCR.
Lane M 1-kb DNA ladder

1. 0.97
2. 1.08
3. 1.09
4. 1.24
5. 0.94
6. 2.07
7. 1.12
8. 1.09
9. 1.64
10. 1.50
11. 1.22
12. 1.92
13. 1.92
14. 1.92
15. 2.74



3. cDNA Normalization

cDNA generated using SMART technology was normalized using Duplex-Specific Nuclease (DSN) normalization (Zhulidov et al., 2004; Shagin et al., 2002). Prior to cloning, the efficiency of normalization was assessed by virtual Northern blot analysis (Franz et al., 1999) comparing the abundance of β-actin and GAPDH in normalized and non-normalized human brain cDNA.

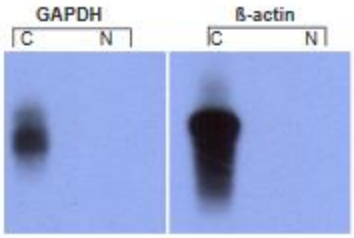


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) HeLa S3 cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of GAPDH and ß-actin were labeled with 32P-dATP and hybridized to the membrane. GenBank Accession numbers: GAPDH, NM_002046 and ß-actin, NM_001101.

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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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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