Certificate of Analysis

Mate & Plate™ Library - Universal Human (Normalized)

Catalog No.	Lot Number
630480	1712612A

Description

This yeast two-hybrid library was constructed from human 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.

A Universal human cDNA library transformed into yeast strain Y187. The library can be readily mated to a MATa GAL4 reporter strain, such as AH109 or Y2HGold (Clontechniques, 2008).

Package Contents

- 5 x 1.0 ml Mate & Plate Library - Universal Human (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

- Mixture of Poly A+ RNAs from a collection of adult human tissues chosen to represent a broad range of expressed genes. Both male and female donors are represented. Modeled after our Human Universal Reference Total RNA (Cat. no. 636538).

Cloning Vector

- pGADT7-RecAB

Cloning Site

- Sfi I A/Sfi I B

Priming Method

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

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Yeast Genotype

- (Y187): MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, met–, URA3 :: $GAL1_{UAS}$-$GAL1_{TATA}$-LacZ, MEL1

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 User Manual (PT4084-1)
- pGADT7-RecAB Vector Information (PT3718-5)

References

Franz, O., Bruchhaus, I. & Roeder, T. Verification of differential gene transcription using virtual northern blotting. *Nucleic Acids Res.* **27,** e3 (1999).

Pretransformed Mate & Plate Libraries. Clontechniques **24**, 26–27 (2008).

Shagin, D. A. *et al.* A novel method for SNP detection using a new duplex-specific nuclease from crab hepatopancreas. *Genome Res.* **12,** 1935–1942 (2002).

Zhulidov, P. A. *et al.* Simple cDNA normalization using kamchatka crab duplex-specific nuclease. *Nucleic Acids Res.* **32,** e37 (2004).

Quality Control Data

1. **Quality Control Data**

Test	Result
Titer (yeast colonies)	>5 x 10^7 cfu/ml
Number of independent clones	4.0 x10^6
Average cDNA size	1.34 kb
cDNA size range*	0.5 - 2.0 kb

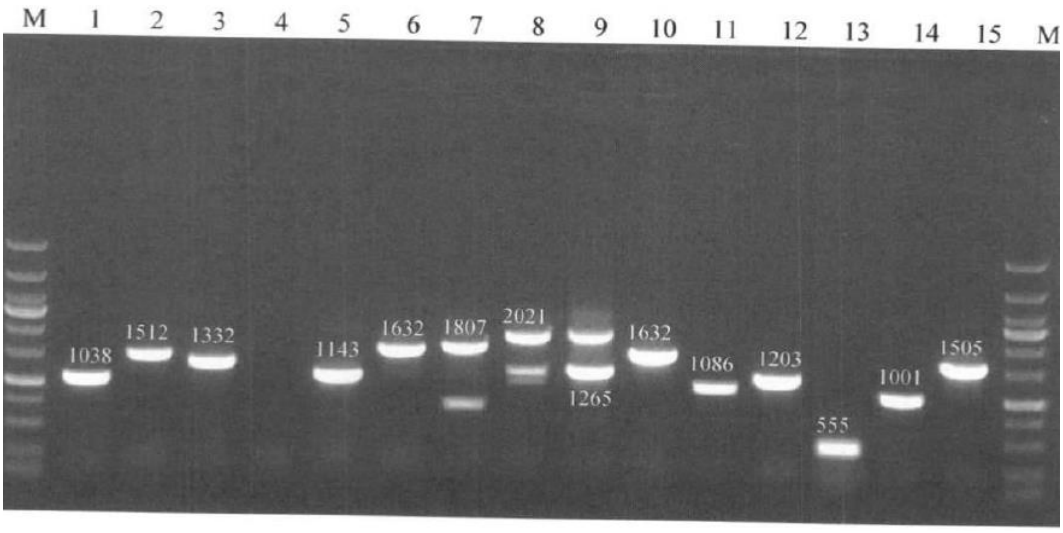
*the cDNA was size-selected by excision from an agarose gel prior to cloning

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).

14 of 15 colonies contained inserts as determined by PCR.

Lane M. 1 kb DNA ladder

1. 1.04 kb
2. 1.51 kb
3. 1.33 kb
4. -----
5. 1.14 kb
6. 1.63 kb
7. 1.81 kb
8. 2.02 kb
9. 1.27 kb
10. 1.63 kb
11. 1.09 kb
12. 1.20 kb
13. 0.56 kb
14. 1.00 kb
15. 1.51 kb



3. cDNA Normalization

cDNA generated using SMART™ technology was normalized using Duplex-Specific Nuclease (DSN) normalization (2, 3). Prior to cloning, the efficiency of normalization was assessed by virtual Northern blot analysis (4) comparing the abundance of GAPDH and ß-actin in normalized and non-normalized human cDNA.

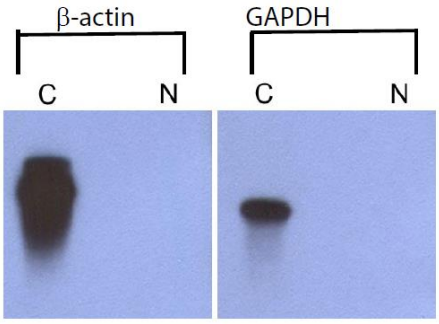


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) Human Universal cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of GAPDH and β-actin were labeled with ^{32}P-dATP and hybridized to the membrane. GenBank Accession numbers: GAPDH, NM_002046 and β-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 purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. 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 prior written approval of Takara Bio USA, Inc.

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TRADEMARKS:

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