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## PRODUCT: Mate & Plate™ Library - Universal Human (Normalized)

**CATALOG No. 630480** 

**LOT NUMBER: 1103585A** 

#### STORAGE CONDITIONS

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

#### SHELF LIFE

1 year from date of receipt under proper storage conditions.

## SHIPPING CONDITIONS

Dry ice (-70°C).

## **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 the Clontech Human Universal Reference Total RNA (Cat. No. 636538).

**CLONING VECTOR:** pGADT7-RecAB

**CLONING SITE: Sfi | A/Sfi | B** 

PRIMING METHOD: Sfi I (dT)<sub>30</sub> primed

YEAST GENOTYPE (Y187): MATa, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4∆, gal80∆, met-, URA3 :: GAL1<sub>UAS</sub>-GAL1<sub>TATA</sub>-LacZ, MEL1

#### 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 (1).

## 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)

## **OTHER**

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

## FOR RESEARCH USE ONLY

## **QUALITY CONTROL DATA**

## 1. Quality Control Data

A. Titer (yeast colonies):  $\geq$ 5 x 10<sup>7</sup> cfu/ml

B. Number of

independent clones: 7.5 x 10<sup>6</sup> C. Average cDNA size: 1.48 kb D. cDNA size range: 0.7 - 2.8 kb

(The cDNA was size-selected by excision from an agarose gel prior to cloning)

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(PA943192)

## **QUALITY CONTROL DATA** continued

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

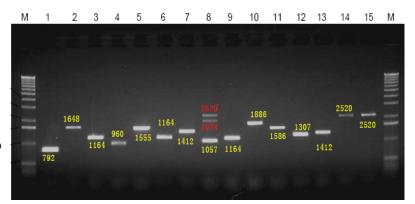
Library Insert Size Screening

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.



- 1. 0.79 kb
- 2. 1.65 kb
- 3. 1.16 kb
- 4. 0.96 kb
- 5. 1.56 kb
- 6. 1.16 kb
- 7. 1.41 kb
- 8. 2.52, 2.08, 1.06 kb
- 9. 1.16 kb
- 10. 1.89 kb
- 11. 1.59 kb
- 12. 1.31 kb
- 13. 1.41 kb
- 14. 2.52 kb
- 15. 2.52 kb



## 3. cDNA Normalization

cDNA generated using SMART<sup>™</sup> 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.

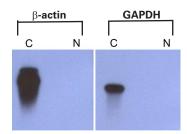


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) Human Universal cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of GAPDH and β-actin were labeled with  $^{32}$ P-dATP and hybridized to the membrane. GenBank Accession numbers: GAPDH, NM\_002046 and β-actin, NM\_001101.

## **REFERENCES**

- 1. Pretransformed Mate & Plate™ Libraries (January 2008) Clontechniques XXIV(1):26–27.
- 2. Zhulidov, P.A., et al. (2004) Nucleic Acids Res. 32:e37.
- 3. Shagin, D.A., et al. (2002) Genom Res. 12:1942-1953.
- 4. Franz, O., et al. (1999) Nucleic Acids Res. 27:e3.

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# **Mate & Plate**<sup>TM</sup> **Library - Universal Human (Normalized)**

CATALOG NO.

630480

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