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

CATALOG No. 630486

LOT NUMBER: 1409412A

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+ RNA isolated from normal, whole brains from 8 male Caucasians, ages: 43–66; cause of death: sudden death.

CLONING VECTOR: pGADT7-RecAB

CLONING SITE: Sfi I A/Sfi I B

PRIMING METHOD: Sfi I (dT)₃₀ primed

YEAST GENOTYPE (Y187): *MAT α* , *ura3-52*, *his3-200*, *ade2-101*, *trp1-901*, *leu2-3, 112*, *gal4 Δ* , *gal80 Δ* , *met-*, *URA3 :: GAL1_{UAS}-GAL1_{TATA}-LacZ*, *MEL1*

DESCRIPTION

This yeast two-hybrid library was constructed from mRNA isolated from human brain tissue and transformed into yeast strain Y187. The cDNA was normalized prior to library construction to reduce the copy number of abundant cDNAs derived from highly represented mRNAs, thereby increasing the representation of low copy number transcripts. The normalization process combines a Duplex-Specific Nuclease (DSN) treatment and SMART™ technology, reduces the number of clones that must be screened in your yeast two-hybrid assay, and facilitates the identification and characterization of novel protein-protein interactions.

The library was transformed into yeast strain Y187 and can be readily mated to a *MAT α* GAL4 reporter strain, such as AH109 or Y2HGOLD (1), for screening.

PACKAGE CONTENTS

- 5 x 1.0 ml Mate & Plate Library - Human Brain (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: | $\geq 5 \times 10^7$ cfu/ml |
| B. Number of independent clones: | 3.2×10^6 |
| C. Average cDNA size: | <u>1.56 kb</u> |
| D. cDNA size range: | <u>0.7 – 3.0 kb</u> |

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

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Clontech

United States/Canada
800.662.2566

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PA913041)

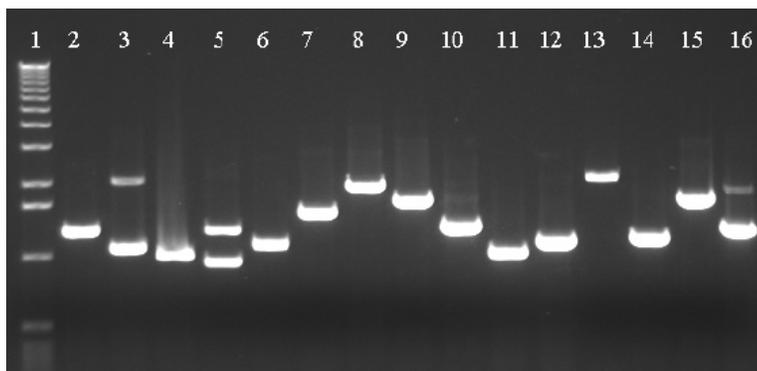
QUALITY CONTROL DATA continued**2. Quality Control Data for the Pretransformed Library in Yeast**

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.

Lane	1.	1 kb DNA ladder
	2.	1.23
	3.	1.05
	4.	1.01
	5.	1.33 + 0.94
	6.	1.13
	7.	1.57
	8.	2.17
	9.	1.81
	10.	1.37
	11.	1.07
	12.	1.18
	13.	2.39
	14.	1.21
	15.	1.83
	16.	1.35

**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 β -actin and GAPDH in normalized and non-normalized human brain cDNA.

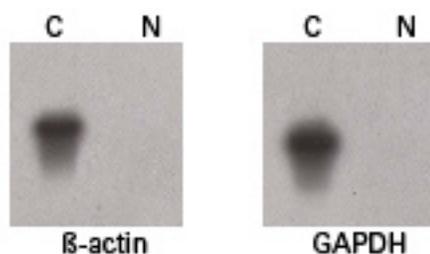


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) human brain cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of β -actin and GAPDH were labeled with 32 P-dATP and hybridized to the membrane. GenBank Accession numbers: β -actin, NM_0011101 and GAPDH, NM_002046.

REFERENCES

1. Pretransformed Mate & Plate™ Libraries (January 2008) *Clontechiques* **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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630486

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Clontech Laboratories, Inc.

A Takara Bio Company

1290 Terra Bella Avenue, Mountain View, CA 94043, USA

U.S. Technical Support: tech@clontech.com

11/20/2015

United States/Canada

Asia Pacific

Europe

Japan

800.662.2566

+1.650.919.7300

+33.(0)1.3904.6880

+81.(0)77.543.6116