Certificate of Analysis

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

CATALOG No. 630486

LOT NUMBER: 1604139A

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**a*** 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:	≥ 5 x 10^7 cfu/ml
B.	Number of independent clones:	3.2 x 10^6
C.	Average cDNA size:	1.56 kb
D.	cDNA size range:	0.7 – 3.0 kb

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

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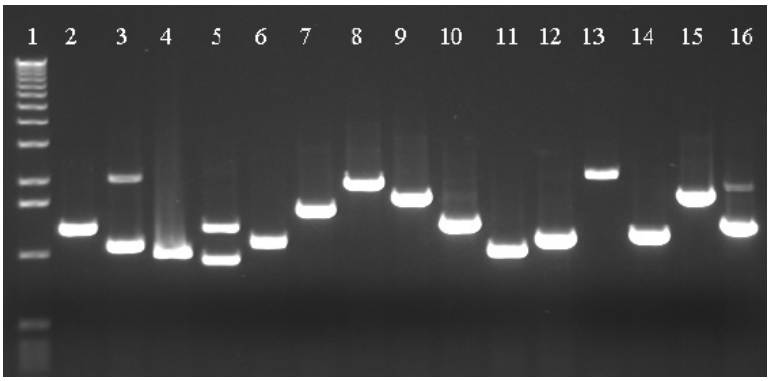
QUALITY CONTROL DATA continued

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.

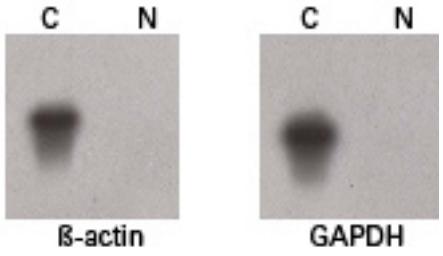


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) human brain cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of β-actin and GAPDH were labeled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

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.

Notice to Purchaser

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