Certificate of Analysis

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

CATALOG No. 630483

LOT NUMBER: 1104643A

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 mouse tissues chosen to represent a broad range of expressed genes. Both male and female animals are represented. Modeled after the Clontech Mouse Universal Reference Total RNA (Cat. No. 636657).

CLONING VECTOR: pGADT7-RecAB

CLONING SITE: Sfi I A/Sfi I B

PRIMING METHOD: Sfi I $(dT)_{30}$ primed

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*

FOR RESEARCH USE ONLY

DESCRIPTION
This yeast two-hybrid library was constructed from mouse 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 mouse 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 (1).

PACKAGE CONTENTS
- 5 x 1.0 ml Mate & Plate Library - Universal Mouse (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)

QUALITY CONTROL DATA

1. Quality Control Data

A.	Titer (yeast colonies):	≥5 x 10^7 cfu/ml
B.	Number of independent clones:	2.8 x 10^6
C.	Average cDNA size:	1.58 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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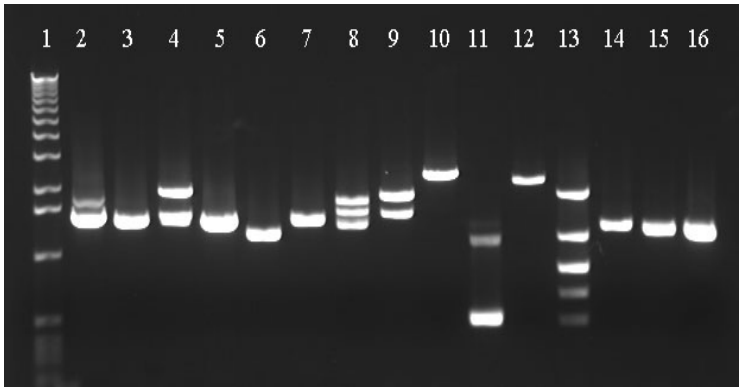
QUALITY CONTROL DATA continued

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. 1.58
3. 1.5
4. 2.14, 1.58
5. 1.5
6. 1.31
7. 1.55
8. 1.97, 1.72, 1.47
9. 2.07, 1.66
10. 2.63
11. 1.24, 0.5
12. 2.45
13. 2.07, 1.28, 0.9
14. 1.47
15. 1.42
16. 1.37



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 mouse cDNA.

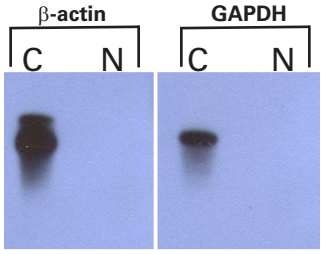


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) Mouse Universal cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of GAPDH and β-actin were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

Notice to Purchaser

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CATALOG NO.

630483

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