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PRODUCT: Mate & Plate™ Library - Mouse Embryonic Stem Cell (Normalized)

CATALOG No. 630484

LOT NUMBER: 8113670

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

Poly A+ RNA isolated from mouse embryonic stem cells (E14TG2A cell line).

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 mouse embryonic stem cells (E14TG2A cell line) 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 - Mouse Embryonic Stem Cell (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: | 2.88×10^6 |
| C. Average cDNA size: | 1.59 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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APPROVED BY: _____



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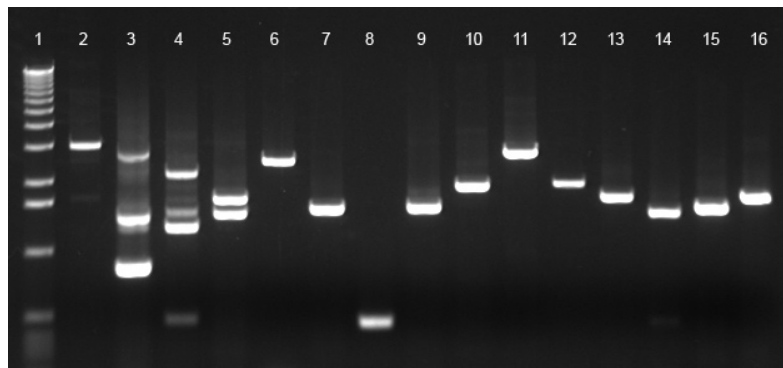
QUALITY CONTROL DATA continued**2. Quality Control Data for the Pretransformed Library in Yeast**

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)

14 of 15 colonies contained inserts as determined by PCR.

Lane	1.	1 kb DNA ladder
	2.	3.16
	3.	1.41 + 0.80
	4.	2.39 + 1.30
	5.	1.80 + 1.49
	6.	2.71
	7.	1.60
	8.	No insert
	9.	1.60
	10.	2.17
	11.	2.99
	12.	2.16
	13.	1.82
	14.	1.54
	15.	1.63
	16.	1.80

**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 mouse embryonic stem cell cDNA (Figure 1).

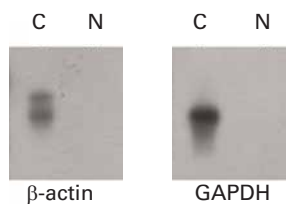


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) mouse embryonic stem cell cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of β -actin and GAPDH were labeled with 32 P-dATP and hybridized to the membrane. GenBank Accession numbers: β -actin, NM_001101. and GAPDH, NM_02406.

QUALITY CONTROL DATA continued

4. Embryonic Stem Cell Confirmation

The mRNA from mouse ES cells expresses 4 pluripotency-related genes, *Nanog*, *Oct-4*, *Klf-4*, and *Sox-2* (Figure 2).

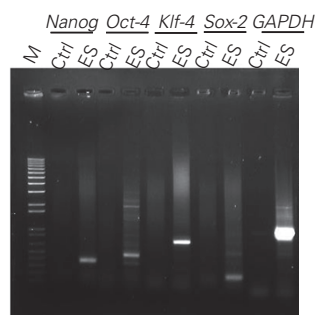


Figure 2. ES cells express pluripotency-related markers. mRNA from embryonic stem cells (ES) (E14TG2A cell line) were analyzed for gene expression markers associated with pluripotent (stem) cells. M, molecular weight marker (1 kb Plus DNA ladder.)

REFERENCES

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

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