Certificate of Analysis

In the interest of conserving resources, we are no longer shipping manuals with products. Please visit **www.clontech.com/manuals** to obtain an electronic version.

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): *MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, met–, URAS :: 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**a*** 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:	≥ 5 x 10^7 cfu/ml
B.	Number of independent clones:	2.88 x 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)

Continued on back of page.

APPROVED BY: _____

(PA913039)

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

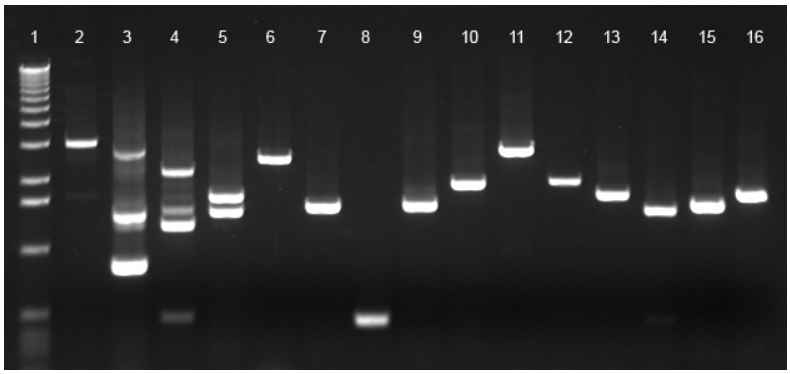
QUALITY CONTROL DATA continued

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).

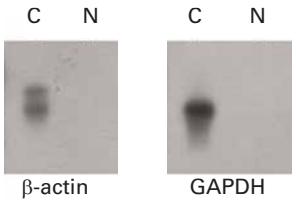


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lanes N) and non-normalized (Lanes C) mouse embryonic stem cell cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of β-actin and GAPDH 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).

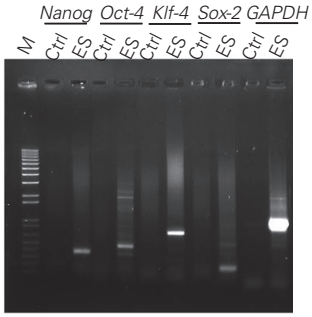


Figure 2. ES cells express pluripotency-related markers. mRNA from embryonic stem cells (ES) (E14TG2A cell line) were analyzed for gene expression markers associated with pluripotent (stem) cells. M, molecular weight marker (1 kb Plus DNA ladder.)

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.