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 - HeLa S3 (Normalized)

CATALOG No. 630479

LOT NUMBER: 1502291A

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
Cervical Adenocarcinoma (HeLa S3; a clonal derivative of the parent HeLa cell line) poly A+ RNA. ATCC No. CCL2.2

CLONING VECTOR: pGADT7-RecAB

CLONING SITE: Sfi I A/Sfi I B

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

DESCRIPTION
This yeast two-hybrid library was constructed from HeLa S3 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.

HeLa S3 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 - HeLa S3 (Normalized)
- 1 x 1.0 ml Mate & Plate Library - Control (pGADT7-T in Y187)

PRODUCT USER MANUAL
User manuals for Clontech products are available for download at www.clontech.com/manuals. The following user manual applies to this product:
- 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 (yeast colonies):	≥5 x 10^7 cfu/ml
B.	Number of independent clones:	3.25 x 10^8
C.	Average cDNA size:	1.43 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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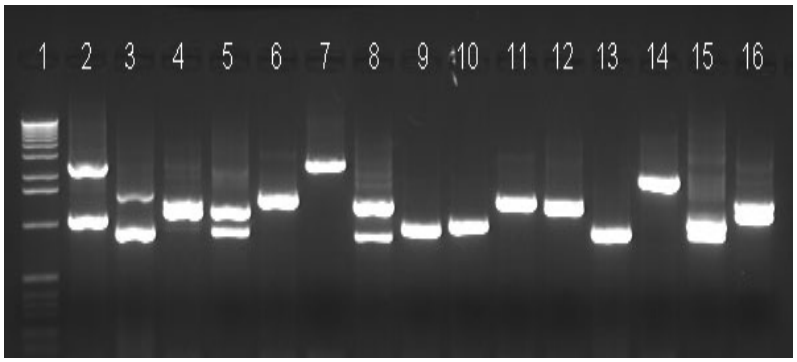
QUALITY CONTROL DATA continued

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)

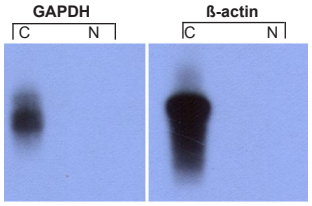
15 of 15 colonies contained inserts as determined by PCR.

Lane
1. 1-kb DNA ladder
2. 2.42, 1.09
3. 0.93
4. 1.29
5. 1.25, 0.96
6. 1.47
7. 2.58
8. 1.33, 0.87
9. 0.96
10. 0.99
11. 1.42
12. 1.38
13. 0.90
14. 1.92
15. 0.99
16. 1.25



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 human HeLa S3 cDNA.



Normalized (Lanes N) and non-normalized (Lanes C) HeLa S3 cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. PCR-amplified probes of GAPDH and ß-actin were labeled with 32P-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):X-Y.
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

Mate & Plate™ Library - HeLa S3 (Normalized)

CATALOG NO.

630479

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