Certificate of Analysis

Mate & Plate™ Library - Universal Arabidopsis (Normalized)

Catalog No.	Lot Number
630487	1811044A

Description

This yeast two-hybrid library was constructed from mRNA isolated from 11 Arabidopsis tissues, mixed in equal quantities 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 MATa GAL4 reporter strain, such as AH109 or Y2HGold (1), for screening.

Package Contents

- 5 x 1.0 ml Mate & Plate Library - Universal Arabidopsis (Normalized)
- 1 x 1.0 ml Mate & Plate Library - Control (pGADT7-T in Y187)

Storage Conditions

- Store all components at –70°C
- Do not refreeze

Shelf Life

- 1 year from date of receipt under proper storage conditions.

mRNA Source

- Mixture of Poly A+ RNAs isolated from 11 *A. colombia* tissues chosen to represent a broad range of expressed genes, and mixed in equal quantities. Please see page 3 for a complete list of source tissues.

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, galΔ, gal80Δ, met–, URA3 :: GAL1$_{UAS}$-GAL1$_{TATA}$-LacZ, MEL1*

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(120616)

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Shipping Conditions

- Dry ice (–70°C)

Product Documents

Documents for our products are available for download at **takarabio.com/manuals**
The following documents apply to this product:

- Matchmaker® Gold Yeast Two-Hybrid User Manual (PT4084-1)
- pGADT7-RecAB Vector Information (PT3718-5)

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.

Quality Control Data

1. Quality Control Data

Test	Result
Titer (yeast colonies)	>5 x 10^7 cfu/ml
Number of independent clones	5.85 x 10^6
Average cDNA size	1.42 kb
cDNA size range*	0.8 - 1.8 kb

*the cDNA was size-selected by excision from an agarose gel prior to cloning

2. Quality Control Data for the Pretransformed Library in Yeast

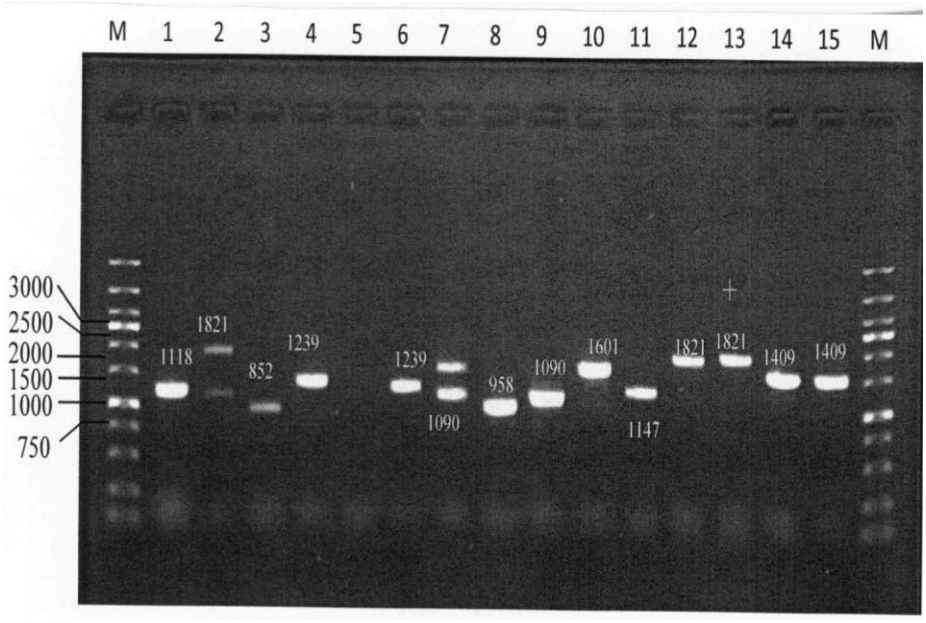
Library Insert Size Screening

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 M. 1 kb DNA ladder

1. 1.12 kb
2. 1.82 kb
3. 0.85 kb
4. 1.24 kb
5. -----
6. 1.24 kb
7. 1.09 kb
8. 0.96 kb
9. 1.09 kb
10. 1.60 kb
11. 1.15 kb
12. 1.82 kb
13. 1.82 kb
14. 1.41 kb
15. 1.41 kb



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 in normalized and non-normalized Arabidopsis cDNA.

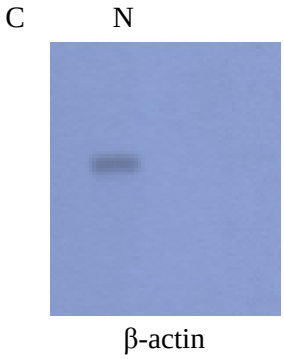


Figure 1. DSN-Normalization reduces the amount of highly abundant transcripts. Normalized (Lane N) and non-normalized (Lane C) Arabidopsis cDNA samples (PCR products) were electrophoresed on a 1.5% agarose gel and transferred to Hybond-N membrane. A PCR-amplified probe for β-actin was labeled with ^{32}P-dATP and hybridized to the membrane. GenBank Accession number: β-actin NM_001101.

mRNA Source

Mixture of Poly A+ RNAs isolated from 11 *A. colombia* tissues, chosen to represent a broad range of expressed genes and mixed in equal quantities:

1–4. Seedlings (intact, including roots). Samples were taken every 4 hr over a 24 hr period, and again at 10 days, from long day or short day grown seedlings. The samples from each treatment were pooled together, for equal representation from each time point.

5. Etiolated seedlings, grown for 5 days in the dark.
6. Open (for 1–2 days) flowers.
7. Buds (closed flowers from different stages).
8. Pollen (unfertilized, emasculated pistils, 1 day after emasculation).
9. Siliques from ALL stages, from very young (3 days after pollination) to full, mature green siliques. No dry siliques were included.
10. Leaves from plants grown in soil, before and after bolting.
11. Stems.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Notice to Purchaser

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630487

NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

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TRADEMARKS:

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