Certificate of Analysis

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PRODUCT: Human Aorta Matchmaker™ cDNA Library

CATALOG No. 638813

LOT NUMBER: 8030090

STORAGE CONDITIONS
- Store library at –70°C.
- Avoid repeated freeze/thaw cycles.

SHELF LIFE
1 year from date of receipt under proper storage conditions

SHIPPING CONDITIONS
Dry ice (–70°C)

mRNA SOURCE
Normal, whole aortas pooled from 38 male/female Caucasians, ages 16–73; cause of death: trauma

NOTE: No further information on the mRNA source was made available to Clontech.

PACKAGE CONTENTS
- 2 x 1.0 ml library culture (in *E. coli* BNN132)
- *Saccharomyces cerevisiae* strain AH109
- *Saccharomyces cerevisiae* strain CG-1945
- Matchmaker™ Two-Hybrid System 3 User Manual (PT3247-1)
- Clontech Yeast Protocols Handbook (PT3024-1)
- pACT2 Vector Information Packet (PT3022-5)

*** TITER:** $\geq 10^8$ cfu (of BNN132)/ml in LB broth & 25% sterile glycerol

CLONING VECTOR: pACT2

CLONING SITE: *Xho* I/*Eco*R I

ADAPTOR SEQUENCE: 5' AATTCGCGGCCGCGTCGAC3'
3' GCGCCGGCGCAGCTG5'

PRIMING METHOD: *Xho* I-$(dT)_{18}$ primed

FOR RESEARCH USE ONLY

QUALITY CONTROL DATA

1. Library Information

A. Estimated % of Colonies with Inserts:	93%
B. Number of Independent Clones:	3.5×10^6
C. Average cDNA Size:	1.7 kb
D. cDNA Size Range:	0.4 – 3.6 kb

(The average cDNA size and size range were determined by examination of an autoradiogram of the cDNA prior to cloning.)

E. Amplification: This library was amplified once in BNN132.

- This pACT2 library was released from a λACT2 library.

* Please retiter the library before use. For library amplification, plate out the library to obtain isolated colonies on each plate (approximately 10^4–10^5 colonies per plate). Plating at a higher density may cause loss of library representation.

APPROVED BY: _____

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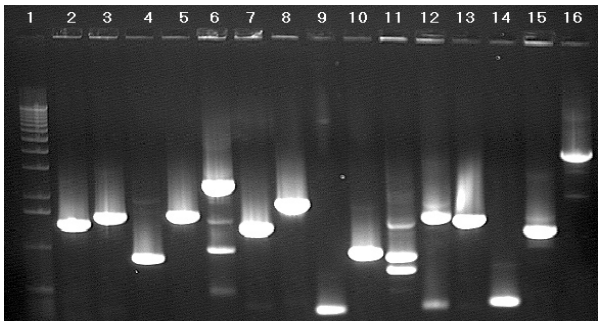
QUALITY CONTROL DATA continued

2. Insert Size Range Analysis

15 colonies were randomly picked and subjected to long-distance PCR using Clontech's Matchmaker™ AD LD-Insert Screening Amplimers (Cat. No. 630433) and Advantage® cDNA PCR Kit (Cat. No. 639101) in a Perkin-Elmer DNA Thermal Cycler.

14 of the 15 colonies contained inserts as determined by PCR.

Lane:
1. 1-kb DNA ladder
2. 1.5 kb
3. 1.6 kb
4. 0.9 kb
5. 1.6 kb
6. 2.6 kb
7. 1.4 kb
8. 2.0 kb
9. no insert
10. 1.0 kb
11. 0.9 kb
12. 1.6 kb
13. 1.5 kb
14. 0.4 kb
15. 1.3 kb
16. 3.5 kb



3. Coprecipitant Usage

This library was constructed using yeast tRNA as a coprecipitant.

☐ yes ☒ no

The library was subjected to PCR screening using yeast-specific 5S rRNA primers. No amplified product was observed.

4. Analysis of Sequence Representation

Sequence representation is evaluted by colony or plaque hybridization using a gene-specific probe. Mammalian libraries are screened with a human β-actin probe which cross-reacts with all mammalian β-actin cDNA. All human cDNA libraries must show a minimum β-actin frequency of 0.10%, and all other mammalian cDNA Libraries must show minimum β-actin frequency of 0.05%. Nonmammalian cDNA libraries are screened with a ubiquitously expressed species-specific probe.

Note: The frequency of β-actin positive clones varies among libraries made with RNA from different tissues and species. A frequency of >0.10% in a human cDNA library suggests a reasonably high probability of finding a rare transcript [Hagen, F. S., et al. (1988) BioTechniques **6**:340–345.] For nonhuman mammalian cDNA libraries, a frequency of 0.05% suggests a reasonably high probability of finding a rare message in those libraries (Clontech observation, unpublished).

The percentage of colonies or plaques to which β-actin hybridized: 0.46%

Note: The β-actin probe may hybridize to other forms of actin.

QUALITY CONTROL DATA continued

5. PCR Screening for Specific Sequences

1 µl of the library lysate was screened by PCR using PCR primers for one or more of the following gene transcripts: β-actin, G3PDH, or transferrin receptor. β-actin and G3PDH represent high-abundance gene transcripts whereas transferrin receptor represents a low-abundance gene transcript. Furthermore, the β-actin primers are designed to amplify a 1.1-kb fragment near the 5' end and thus provide a stringent test for detecting the presence of full-length transcripts. (Note: Species-specific primer sets are used for human, mouse, and rat cDNA libraries; human primer sets are used for other mammalian cDNA libraries.)

The presence of the following 3 sequences were determined using PCR.

β-actin

G3PDH

transferrin receptor