

Matchmaker™ Insert Check PCR Mix Protocol-at-a-Glance

(PT4102-2)

Matchmaker Insert Check PCR Mix 1 (Cat. No. 630496) is designed to be used with the Matchmaker Gold Yeast One-Hybrid Library Screening System (Cat. No. 630491) to confirm the integration site of the pAbAi vector containing your DNA sequence of interest.

Matchmaker Insert Check PCR Mix 2 (Cat. No. 630497) is designed to be used with our Matchmaker Gold Yeast One- and Two-Hybrid Screening Systems (Cat. Nos. 630491 and 630489, respectively) to analyze cDNA inserts in library prey vectors following yeast one- and two-hybrid screening. This mix is compatible with GAL4 AD libraries constructed in the following vectors: pGADT7, pGADT7-Rec, pGADT7-Rec2, pGAD424, pACT, pACT2, pGAD GH, pGAD GL, and pGAD10.

List of Components

Store at -20°C .

- 2X Matchmaker Insert Check PCR Mix 1 or 2

Protocol:

1. For each yeast colony to be analyzed, add 25 μl of PCR-grade H_2O to a PCR tube.
2. Lightly touch a well isolated yeast colony with the end of a clean toothpick to obtain a **very small** amount of yeast. Place the tip of the toothpick into one of the tubes of PCR-grade H_2O from Step 1 and stir to disperse the yeast. Repeat for each yeast colony to be analyzed.



Note: Do not use the entire colony, as too many cells will inhibit the reaction. If the water becomes cloudy after you add the cells, you have added too many.

3. Aliquot 25 μl of Matchmaker Insert Check PCR Mix into each tube. Mix by pipetting up and down 3–5 times.

Each reaction tube will now contain a total of 50 μl :

25 μl Matchmaker Insert Check PCR Mix
25 μl H_2O /yeast
 50 μl Total

4. Begin thermal cycling using the appropriate cycling parameters:

| | <u>For Mix 1</u> | <u>For Mix 2</u> |
|-----------|---|---------------------------------------|
| 1 cycle | 95°C, 1 min | 1 cycle 94°C, 1 min |
| 30 cycles | 98°C, 10 sec 55°C, 30 sec 68°C, 2 min | 30 cycles 98°C, 10 sec 68°C, 3 min |

5. Analyze 5 μl of each reaction by electrophoresis on a 1% agarose/EtBr Gel, along with an appropriate DNA size marker.



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

(PR912682; published 30 January 2009)

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PCR:

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