PCR Amplification Protocol for the NIA 15K set

Introduction:

Amplification of the cDNA clones represented in the 15k NIA clone set is a challenge. To effectively produce microarrays from these clones, the amplifications should be efficient, low cost, and reproducible with a low rate of cross contamination. To this end, we have used the protocols developed by TIGR (Hedge, *et al.* Biotechniques 29:548-562. Sept. 2000) with a few minor modifications.

Materials:

- Fresh overnight cultures grown in 96-well plates. We have found that as cultures ‘age’ when stored at 4 °C, the uniform loading of replicator slot pins is affected.
- 1 µl or 5 µl slot pin replicators (*V & P Scientific. Vp408S5, VP408S10 www.vp-scientific.com*)
- Primers: (These were developed by TIGR and can be used on most vectors which have M13 sequences)
  - M13 FWD: 5' -GTTTTCCCAGTCACGACGTTG-3'
  - M13 REV: 5'-TGAGCGGATAACAATTTCACACAG-3'
- Standard PCR Reagents
- 96-well PCR plates (*Fisher 05-500-63*)
- 96-well u-bottom plates (*Costar 3795*)
- Thermal cycler (*MJ Research PTC-2225-we have 3 tetrads and typically run 12-96-well PCR plates at a time*)
- Microseal A Mats for sealing PCR plates (*MJ Research MSA 5001*)

Methods:

**DAY 1:**

**INOCULATION**

- 165 µl of LB containing 100 µg/ml AMP is dispensed using a Labsystems Multidrop DW into 12 96-well U-bottom plates (*Costar 3795*).
- A 1 µl slot pin replicator (*V&P Scientific*) is used to inoculate each 96well plate, and the plate is covered with Qiagen Air Pore tape sheets (#19571) and incubated overnight at 37°C with shaking for 16 – 18 hours.

**DAY 2:**
LYSIS

- 50µl of milliQ H2O is dispensed into 96-well PCR plates (*Fisher*) and placed on ice (to reduce loss of volume from evaporation) using a Labsystems Multidrop DW.

- Overnight cultures are inoculated into the PCR plates containing MilliQ water using a 10µl slot pin replicator (*Note: we have found that we needed a larger inoculum in the lysis step than that recommended by TIGR.*)

- The PCR plates are covered with a thermal seal (MJ Research-Microseal A film MSA 5001) and incubated at 95°C for 10 minutes.

- Cellular debris from the lysed cells are pelleted by centrifugation at 1200xg for 4 minutes in a centrifuge equipped with microplate carriers.

PCR

- 20µl of MilliQ H2O is dispensed into 96-well PCR plates and placed on ice (to reduce loss of volume from evaporation) using a Labsystems Multidrop DW.

- A 5µl slot pin replicator is used to transfer approx. 5–10µl (2x inoculation of the 5µl or a single inoculation with the 10µl slot pin replicator) of the lysate into the 20µl of Milli-Q water and the inoculum is placed on ice (*Note: lysates may be prepared in advance and stored at –20°C.*)

- A Master mix is made for the 12-96 well plates as follows (each well houses one reaction):

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X PCR buffer</td>
<td>11</td>
</tr>
<tr>
<td>dNTP’s</td>
<td>0.9</td>
</tr>
<tr>
<td>(M13F/M13R)</td>
<td>0.256</td>
</tr>
<tr>
<td>Taq</td>
<td>5</td>
</tr>
<tr>
<td>H2O(milli-Q)</td>
<td>76</td>
</tr>
</tbody>
</table>

  **90 µl total**

  **Notes:**
  - Stock dNTPs are 25 mM each dNTP.
  - Primer concentration is 20 pmol of each primer/reaction.
  - Units/reaction of Taq required will vary by manufacturer. We have had good success with Taq from Promega as well as Stratagene (Yieldace).
  - If the Lab Drop dispenser is used, add a 1.5 plate dead volume due to losses in the tubing.
  - Multiply the per reaction volumes by the number of reactions planned + 2 reactions to account for pipette variation.
90µl of master mix is added to each well containing the bacterial lysate to give a final reaction volume of 110µl/well using the Labsystems Multidrop.

The PCR plates are covered with microseal A (MJ Research) and amplified by using the following conditions:

- Initial denaturation step of 3 minutes at 96°C
- 40 cycles: 95°C 30 sec
  55°C 30 sec
  72°C 2.5 minutes (product sizes range up to 3kb)
- Final extension for 7 minutes at 72°C
- Soak at 4°C

PCR products are analyzed by agarose gel electrophoresis. We routinely run out 5µl of product on 1.5% agarose gels to check for yield and multiple products. PCR reactions are quantitated based on molecular mass markers (BIORAD EZ Load Precision Molecular Mass Ruler). Yields ranged from 400 ng to 20 µg/rxn with a median yield of 10 µg. Gel images from all 15,000 clones in the NIA set can be found on the VMSR website:


Contact:

Vicky Amann, Lab Manager
Vanderbilt Microarray Shared Resource
Vanderbilt University
vicky.amann@vanderbilt.edu
http://array.mc.vanderbilt.edu