

PCR PROTOCOL FOR cDNA ARRAYS ON MEMBRANES

Purpose: to amplify insert DNA from purified plasmid DNA derived from bacterial plasmid libraries. These products are to be used to make spotted cDNA microarrays, spotting onto nylon membranes.

Materials Needed:

- ❑ Low profile polystyrene 96-well PCR plates (Marsh Bioproducts, cat# AB-0800-150)
- ❑ PCR plate sealers (Apogent Discoveries, cat# 1044-39-4)
- ❑ 12.5ul Matrix barrier tips (Apogent Discoveries, cat # 7635)
- ❑ 1250ul Matrix barrier tips (Apogent Discoveries, cat# 8055)
- ❑ DEPC H₂O
- ❑ Taq DNA Polymerase (Promega, cat# M1668)
- ❑ 10X PCR Buffer (included with Promega Taq)
- ❑ 25mM MgCl₂ (included with Promega Taq)
- ❑ dNTP's 100mM (Invitrogen, cat# 10297-018)
- ❑ Forward M-13 Primer (Research Genetics, cat# custom oligos)
- ❑ Reverse M-13 Primer (Research Genetics, cat# custom oligos)
- ❑ 20%/0.025mg/ml Sucrose/Cresol (Cresol, Sigma-Aldrich, cat# 114480)
- ❑ MJ Research Tetrad PCR machine (MJ Research, cat# PTC-225)
- ❑ Matrix 12.5 ul pipettor (Apogent Discoveries, cat # 2019)
- ❑ Matrix 1250 ul pipettor (Apogent Discoveries, cat # 2004)

Procedure: use techniques compatible with PCR quality levels including the use of barrier tips for all pipetting

Preparation:

1. This protocol is written for amplifying one 96-well plate of purified plasmid DNA. It can be scaled up accordingly. A comfortable processing volume is 8 X 96-well plates per day/scientist, based on two tetrad thermal cycler availability and same day gel analysis.
2. Thaw all reagents on ice, leaving Taq Polymerase @ -20 °C until needed.
3. Thaw purified plasmid DNA templates to room temperature, and vortex lightly before use.
4. Resuspend primers in DEPC water to a final concentration of 120uM.
5. Prepare enough master mix to be distributed between PCR plates.

PCR Master Mix (for one plate):

<u>REAGENT</u>	<u>VOLUME ADDED</u>	<u>FINAL CONCENTRATION</u>
DEPC water	8.26 ml	
Sucrose/Cresol	4.8 ml	20%/2.5 mg/ml
PCR Buffer	1.6 ml	1X
MgCl ₂	960 ul	1.5mM
dNTP's	32 ul of each	0.2mM
RG Forward Primer	66.7 ul	0.5uM
RG Reverse Primer	66.7 ul	0.5uM
Taq DNA Polymerase	120 ul	0.0375U/ul

1. Distribute 160 ul of PCR Master Mix into each well of a 96 well thermal cycler plate (thin walled) using a multi-channel pipettor. One way to configure this is to draw 1120 ul into an 8-channel pipettor using 1250 ul barrier tips and dispensing into 6 rows before refilling, expelling final aliquot to account for pipet variability.
2. Inoculate 3ul from each well of the DNA plasmid master plates. One way to configure this is to draw 3 ul into a 12-channel pipettor using 12.5 ul barrier tips. Save the tips, in proper orientation, for reuse in gel analysis.
3. Perform PCR reaction according to the following cycling program, disabling the heated lid:

1	94°C	2 min	Initial Denaturation
2	94°C	1 min	Cycle Denaturation
3	55°C	1 min	Primer Annealing
4	72°C	2.5 min	Primer Extension
5	Go to Step 2	32 Times	Cycles
6	72°C	10 min	Substrate Clearance
7	4°C	Hold	Storage

Total Time~3.5 hrs

Comments:

1. This varies from other PCR protocols used for cDNA production. It is not recommended for preparation of glass slides.
2. This procedure is also compatible with Invitrogen taq, although the MgCl₂ from this company comes as 50mM.

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For frequently asked questions go to the following address:
<http://www.grc.nia.nih.gov/branches/rrb/dna/protocolFAQs.htm>

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