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Automate front-end tasks using the BioRobot® 3000 for microarray fabrication and streamlined workflow

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A BioRobot® 3000 workstation was used to process 4 x 96 cDNA clones in parallel for a high-throughput microarray project to analyze gene expression profiles in mice. Bacterial cDNA clones were propagated in 96-well format and insert sequences were amplified to provide DNA for microarray spotting. Automation of clone propagation, PCR setup and cleanup, cherry picking, and sample rearray results in high yields of pure PCR products, ready to use in microarray spotting.

Microarray technology has revolutionized genomic research, allowing scientists to monitor the expression profiles of thousands of genes in parallel. A microarray consists of a nylon membrane or glass-derivative slide, onto which DNA sequences are spotted (1).

DNA probes immobilized as spots on microarray slides are cDNAs, gene fragments (ESTs), or oligonucleotides corresponding to known genes or putative open reading frames (ORFs). These arrays are usually hybridized with fluorescently labeled targets synthesized from cellular RNA. The widespread use of microarray technology means that university core facilities such as BIOGEM now require cost-efficient automated solutions to increase sample throughput and reduce handling variability (Figure 1).

The most basic type of experiment compares mRNA abundance in two target samples, a control mRNA and a test mRNA. During hybridization, both targets bind to complementary sequences on the microarray. The microarray is washed to remove nonspecific hybridization and then dried. Hybridization is detected by laser excitation of the fluorescently labeled target sequences. Comparing the intensities of the signals from individual spots shows the relative expression levels of genes in both test and control populations (2). ▶

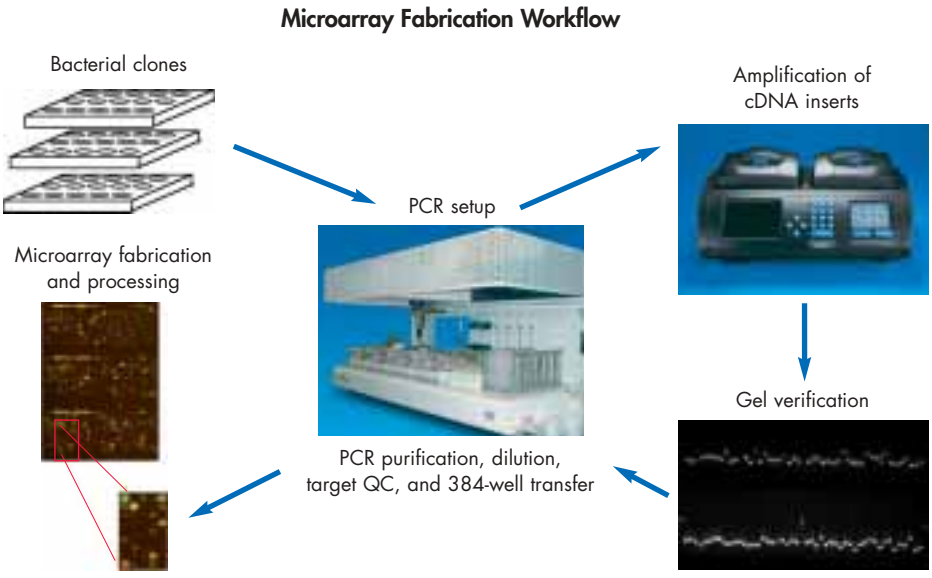

Figure 1 *A typical microarray fabrication procedure involves replication of bacterial clones, amplification of cDNA inserts, verification, purification, and dessication of the PCR amplicons. DNA is then resuspended at a concentration appropriate for microarray spotting.*

The cDNAs spotted onto microarrays are typically PCR products amplified from plasmid DNA purified from cDNA clones. The recommended concentration for spotting on aminosilane and poly-L-lysine slides is 200–400 fmol/µl. Lower concentrations result in weak signals while higher concentrations can cause smearing and create hybridization artifacts. Microarray hybridizations with unpurified PCR products give poor results due to carryover of salts, primers, and partial PCR products. Consequently, PCR amplification and cleanup strategies for array fabrication are optimized to maximize DNA yield and purity.

The precise liquid handling and robotic handling of the BioRobot 3000 workstation are ideally suited for automated purification of plasmid DNA, subsequent PCR setup, and reaction cleanup. High-throughput PCR projects such as microarray analysis rely on highly standardized purification and reaction setup procedures. Errors in front-end processes, such as PCR setup, will be amplified in downstream applications, such as amplification and hybridization, and may produce meaningless data. Microarray spotting is performed by highly specialized instruments that usually require purified DNA in 384-well plates. The robotic handling system of the BioRobot 3000 and the disposable tips of the pipetting system provide the precision and protection from carryover required to accurately rearray samples from 96- to 384-well format.

Materials and methods

A commercially available mouse library containing 8734 clones was used to generate a clone set. Bacterial clones containing cDNA inserts for growth and replication were inoculated into 96-well plates containing selective media and grown overnight with agitation at 37°C. PCR (150 µl each) was set up on the BioRobot 3000 workstation in 96-well microplates using a customized protocol within QIAsoft™, the BioRobot Operating System software. PCR cycling was performed using MJ Research thermal cyclers.

A 10 µl aliquot of each reaction was analyzed on a 0.8% agarose gel and stained with ethidium bromide (Figure 2). The yield of PCR product was assayed using a commercially available dsDNA quantification kit and a microplate fluorescence reader. Bacterial clones that gave no amplification product were cherry-picked using the BioRobot 3000 and inoculated into fresh media in 96-well plates for repeated growth and PCR amplification. Amplicons were purified using the QIAquick® 96 PCR

Evaluation of PCR Products

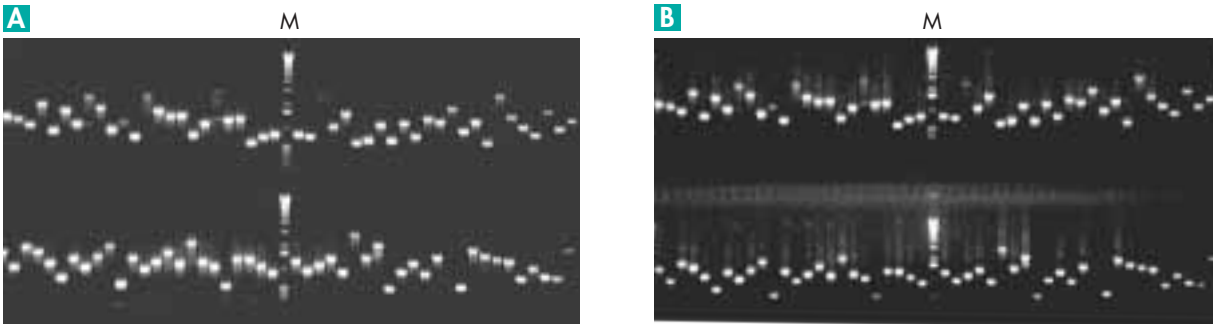

Figure 2 PCR products from a 96-well plate were separated by agarose gel electrophoresis. A Before purification using the QIAquick 96 PCR Purification Kit. B After purification using the QIAquick 96 PCR Purification Kit. M: Molecular weight markers.

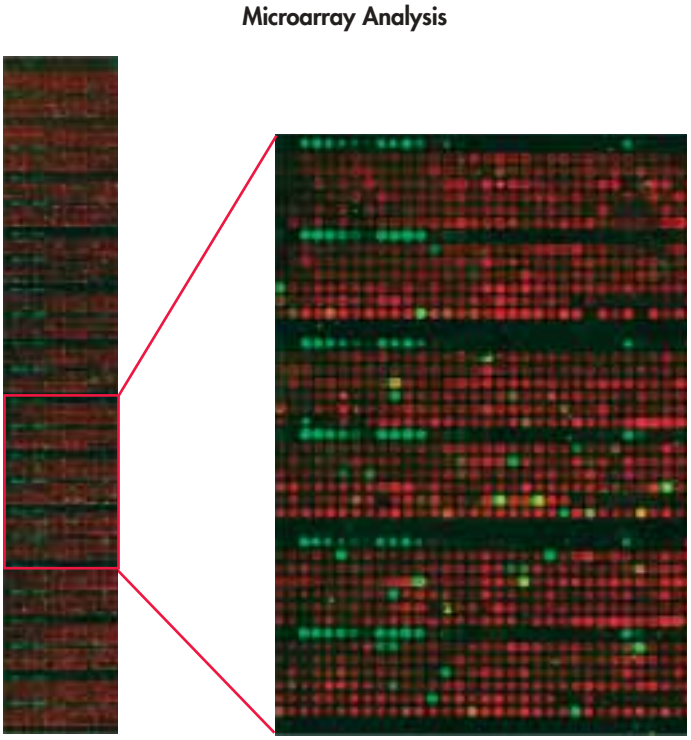

Figure 3 *Approximately 10,000 cDNA clones derived from the mouse clone set and controls were printed on Type 7 reflective slides. Fluorescent targets were synthesized from mouse spleen and control vector sequence RNAs and used to screen the microarray. The enlarged area shows signal color range when using cyanine targets, Cy5 amplicon and Cy3 spleen. Green, red, and yellow spots indicate positive hybridization with the labeled target. Green (Cy3) and red (Cy5) spots indicate that the genes are expressed differentially.*

Purification Kit and an automated protocol on the BioRobot 3000 workstation. QIAquick purified DNA was then rearrayed from 96-well plates to 384-well plates on the BioRobot 3000 workstation prior to spotting (Figure 1).

One microgram each of mouse spleen poly A+ RNA and a synthetic RNA target amplicon created from plasmid vector sequence were reverse transcribed and fluorescently labeled with Cy®3-dCTP and Cy5-dCTP as previously described (1). Remaining RNA was hydrolyzed by addition of 1 µl of 10 M NaOH and subsequent incubation at 37°C for 15 minutes, followed by neutralization. cDNA targets were purified using the QIAquick PCR Purification Kit. Typically, 20% of each reaction was used per hybridization.

Approximately 10,000 PCR products were amplified from the mouse clone set and control cDNA clones. QIAquick purified products were printed on Type 7 reflective slides using a commercially available microarray spotter (Molecular Dynamics). cDNA targets were lyophilized and redissolved in 32 µl hybridization buffer before hybridization for 14–18 hours at 42°C. The microarrays were washed at room temperature, briefly rinsed with water, and dried by centrifugation. Microarrays were scanned using an automated scanner (Molecular Dynamics).

Results and discussion

Amplicon yield and quality

Representative amplifications performed in 96-well format are shown in Figure 2. Most samples gave a single amplicon at high concentration. The quality of QIAquick purified DNA was high and DNA was recovered efficiently.

Of approximately 10,000 samples tested, 8356 samples gave single amplicons. Of these, 7997 samples gave high concentrations of PCR products. 293 samples gave multiple bands and 85 samples failed to give any PCR product. The cDNA clones that generated multiple bands were rearrayed and inoculated for growth using the BioRobot 3000 and amplified with a nested primer set (data not shown). Most samples still gave multiple bands, indicating that these cDNAs represent mixed clones. This indicates a very low rate of cross-contamination for amplification of a large clone set. This is due largely to automation of the array fabrication process using a BioRobot 3000 workstation. ▶

References

1. *Schena, M., Shalon, D., Davis, R.W. and Brown, P.O. (1995) Quantitative monitoring of gene expression patterns with complementary DNA microarray. Science* ***270****, 467.*
2. *Hegde, P. et al. (2000) A concise guide to cDNA microarray analysis. BioTechniques* ***29****, 548.*
3. *Verdun et al. (2002) submitted.*

Representative DNA samples from the entire set of amplifications were quantified. The mean yield per clone was 4955 ng dsDNA. DNAs used for printing were resuspended at a concentration of approximately 200 µg/ml in 25 µl 50% DMSO. Using 200 nl of this solution per spot enables printing of approximately 3000 microarray slides, even considering sample evaporation and other printing variables.

Microarray performance

PCR products amplified using this fabrication scheme generated strong specific signals in a microarray experiment (Figure 3). We printed this set of amplicons representing approximately 10^4 mouse cDNA clones and appropriate controls on a reflective slide. Fluorescent probes synthesized from mouse spleen poly A^+ and control amplicon RNAs were used to screen the microarray by hybridization (Figure 3). Positive hybridization of the labeled targets to the printed PCR products is indicated by green (Cy3), red (Cy5), and yellow fluorescent spots. The enlarged area shows the signal color range observed when using cyanine targets, Cy5 control RNA sequence amplicon and Cy3 spleen. Green and red spots indicate differential hybridization of the targets. The control amplicon hybridization represents quality control of the array as every probe is expected to hybridize to the target. Lack of hybridization indicated a poor probe and data from this spot were not counted. Generally, strong hybridization signals were observed indicating that the quality of the probes was good and that use of the BioRobot 3000 workstation was successful.

Conclusions

- Amplifying large clone collections for the production of microarrays requires minimal cross-contamination and reduction of sources of error due to manual intervention.
- Standardized processing using the BioRobot 3000 workstation together with QIAquick chemistry results in high yields of pure PCR products at concentrations ideal for microarray spotting.
- High yields of PCR product result in a greater number of arrays per PCR and a resultant reduction in time and cost per project. ■

Ordering Information

Product	Contents	Cat. No.
BioRobot 3000	System includes: custom-designed robotic workstation comprised of 2–4 dilutor units, computer controlled shaker and vacuum pump, vacuum manifold, QIAsoft 4.0 Operating System , computer, 1 year warranty on parts and labor. Workstations of various sizes are available.	900400
QIAquick 96 PCR Purification Kit	For purification of 24 x 96 PCR products: 24 QIAquick 96 Plates, Buffers, Collection Microtubes (1.2 ml), Caps	28183
Related product for faster liquid-handling and rearray		
BioRobot RapidPlate	System includes: robotic system, controller unit, software, pre-programmed protocols for reaction setup, 1 year warranty on parts and labor	9000490