



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 (1). 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 glass-slide or nylon membrane, onto which DNA is spotted (2).

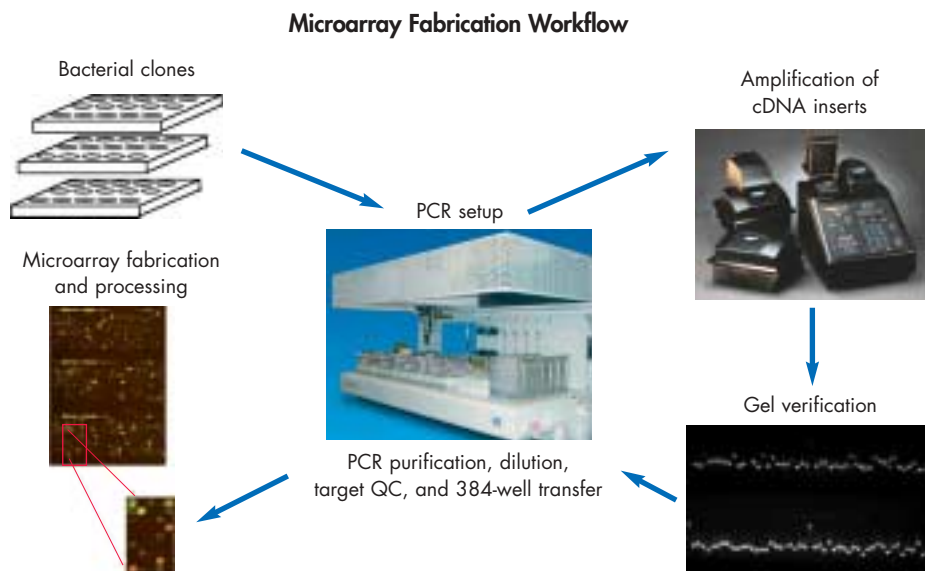


Figure 1. A typical microarray fabrication procedure involves replication of bacterial clones, amplification of cDNA inserts, verification, purification, and dessication of the PCR amplicons. DNA is then resuspended at a concentration appropriate for microarray spotting.



Microarray Analysis

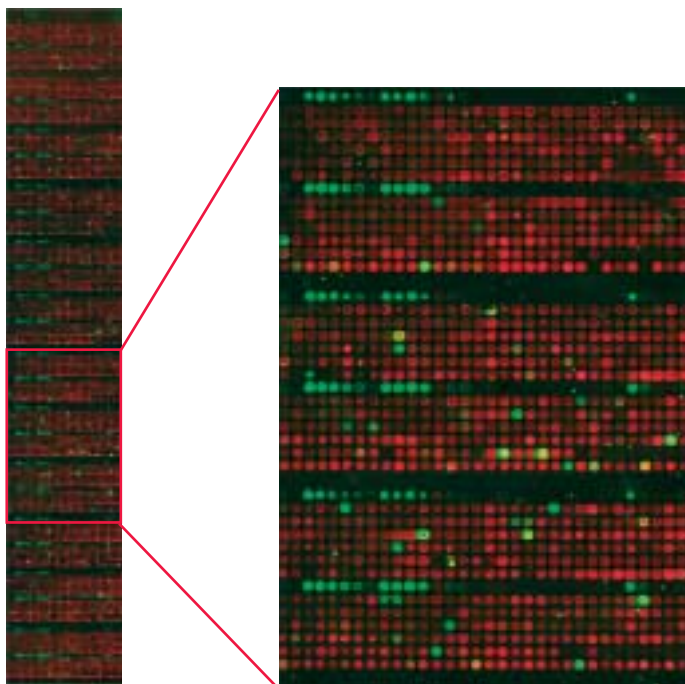


Figure 3. Approximately 10,000 cDNA clones and controls were printed on Type 7 reflective slides. Fluorescent targets were synthesized from mouse spleen and control vector sequence RNAs and used to screen the microarray. The enlarged area shows signal color range when using cyanine targets, Cy5 amplicon 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.

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.



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 rearranged 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.

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.

