Re-use of a stripped cDNA microarray

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The biochip is a new category of developing technology, characterised by expedient, large scale, highly automatic and sensitive detection of biological information such as DNA, RNA and protein. At present, the gene chip is the most common of the biological chips, the gene expression profiling chip (complementary DNA [cDNA] microarray) being used most widely.[1] This technique makes it possible for gene expression to be studied at the whole genome level so as to investigate the functions of the genes. Currently, although the cDNA microarray is an advanced technology, high cost is the main restriction, and this hampers its further application. During studies using super-amine modified glass slides (Corning), we have found that probes hybridised on the chip can be stripped off and the chip reused.

The SH-SY5Y cell line used in this study was a kind gift from Dr Zhang (Chinese Academy of Medical Sciences). Fetal bovine serum was purchased from Gibco. Cell culture medium (RPMI 1640) and guanidine thiocyanate were purchased from Sigma. All other chemicals and reagents, reverse transcriptase AMV, *Thermus aquaticus* (*Taq*) DNA polymerase, and T4 DNA ligase were obtained from TaKaRa.

The SH-SY5Y cell line was maintained in a 5% CO_2 incubator in RPMI 1640 medium containing 15% fetal calf serum, 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were examined twice a week and seeded when 80% confluent.

Preparation of target gene fragments for the cDNA microarray began by selecting clones from a library of SH-SY5Y cell fragments constructed using the restriction display-polymerase chain reaction (RD-PCR) technique[2] and then amplified with the templates of plasmids and plasmid primers thus: primer A (5′-GTAAAACGACGGCCAGT-3′); primer B (5′-CAGGAAACAGCTATGAC-3′).

After precipitation by CTAB,[3] they were dissolved in 10 µL water, the DNA concentration was determined by spectrophotometry and then adjusted to 0.3 mg/mL with DMSO and water.

cDNA microarrays were produced by spotting target gene segments on special glass slides (Corning) using a Cartesian 5500 MicroArrayer, then the DNA of the target genes was cross-linked by ultraviolet irradiation, baked at 80°C for 2 h and maintained in closed containers until used.

Total RNA from the SH-SY5Y cells was extracted using a method previously described.[2] RNA concentration was determined using a Beckman DU530 UV spectrometer. 20µg total RNA was added to the labelling reaction, with 5 µL dATP, dGTP and dTTP (10 mmol/L) and 1 µL dCTP (10 mmol/L), 1.5 µL cy3-dCTP (1 mmol/L), 2 µg oligo(dT_{18}), 15 U AMV, 20 U RNase and water to a total volume of 50 µL.

The sample was incubated at 42°C for 1 h and, after purification with DNA purification kit (Takara), the purified and labelled DNA was dissolved in the prehybridisation solution (5×SSC, 0.2% SDS and 25% formamide).

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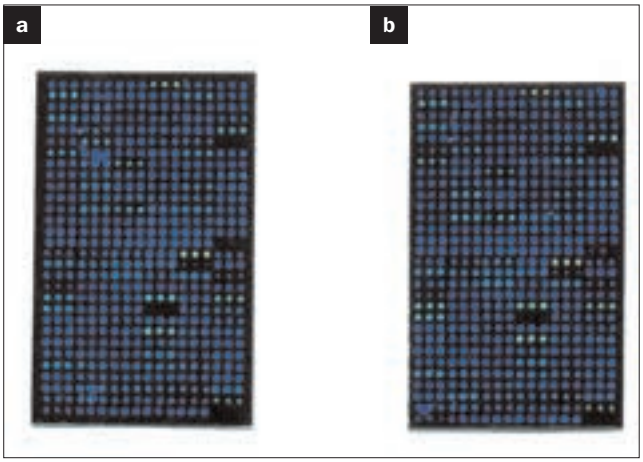
Fig.1. Scanning for hybridisation: (a) first round, (b) third round.

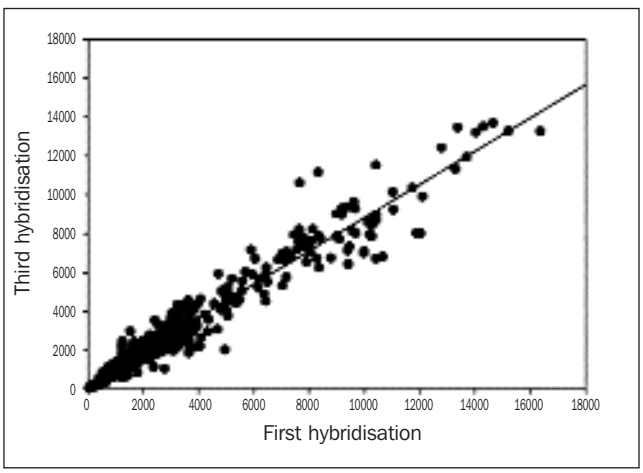


The hybridisation mixture was denatured at 95°C for 5 mins, centrifuged and cooled down. 3.5 µL hybridisation mixture was added to the surface of microarray, which had been immersed in prehybridisation medium for 45 min. After hybridisation for 16 h, the microarray was washed in 2×SSC/0.1% SDS, 0.1×SSC/0.1% SDS, 0.1×SSC, H_2O and alcohol, successively, then the chip was desiccated at room temperature. Scanning was performed using a Scanarray Lite (GSI Lumonics Co).

The chip was incubated in stripping solution preheated to 70°C for 30 mins (x2) then washed in water, 2×SSC, water and alcohol in turn. The results of washing were evaluated by chip scanning and the above procedure repeated if this showed that the probes had not been washed off completely, to make sure that chip could be used in the next experiment.

The result of the first hybridisation of cDNA (Cy3-dCTP labelled) on the microarray (scanning conditions: 96% laser energy, 70% PMT) is shown in Figure 1a. After the stringent probe stripping protocol, no positive signals were detected on the chip, indicating the efficiency of the stripping procedure. Further rounds of hybridisation with the same procedures were carried out to detect whether the cDNA microarray could withstand several rounds of hybridisation

Fig. 2. Scatter diagram showing correlation between the results of the first- and third-round hybridisations (regression line equation: y=236 + 0.856x).



and probe stripping. The third-round hybridisation result is shown in Figure 1b. The results show, as expected, that positive signals were observed and the intensity of the signals correlated directly with the first round (using the same scanning conditions), without significant increase in background.

Analysis of the scanning for the two hybridisations was conducted with SPSS10 software. Average fluorescence intensity of each point was calculated and the results shown in Figure 2. Correlation coefficient: 0.970, $P<0.001$. Regression line equation: $y=236 + 0.856x$.

More and more attention has been focused upon microarray technology because of the advantages of parallel analysis, expediency and sensitivity. Probe purification and the microarray preparation are the critical steps of the technique. Current commercial microarrays are designed for single use only, which increase the cost of microarrays. There are two ways to lower microarray cost, namely increasing the products efficiency and making microarrays re-usable. Here, we focused on a procedure that makes it possible to re-use microarrays.

We stripped hybridised probes from the chip with a stripping solution. The principle of this step is that the Tm of the probes was first reduced in the presence of the denaturant (50% formamide) and then the probes were washed off at 70°C. In our experience, two washings (30 min) in the stripping solution can clear all the probes and no signals from the previous hybridisation (or noise signals) were observed using 96% laser energy, indicating that re-use in the next hybridisation is practicable.

Accuracy and precision are indispensable for a chip to be re-used. On statistical analysis, significant correlation was observed between results of the first and the third hybridisation (correlation coefficient: 0.970, $P<0.001$). However, there would appear to be a 5% loss of efficiency with each round. A number of factors affect hybridisation, such as the extent of hybridisation washing, and further study of these is required. In the present study, the same chip was used up to three times and the experimental results were uniform.

In summary, we report a new technique for stripping hybridised microarrays. The protocol uses a higher concentration of formamide and high temperature to strip off the probe without causing any damage to the microarray. Results indicated that, after the third round of hybridisation and stripping, there was no visible increase in background noise, with a satisfactory correlation coefficient. □

References

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