Benchmark

Room-Temperature Hybridization of Target DNA with Microarrays in Concentrated Solutions of Guanidine Thiocyanate

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The remarkable development of microarray-based automated techniques in recent years has allowed massively parallel analysis of DNA samples. There are two major applications of DNA microarrays in the life sciences: gene expression profiling (1,2) and gene mutation analysis (3,4). Typically, there are three major steps involved in DNA chip-based mutation detection. First, the nucleic acids are extracted from body fluids or tissue, then the target sequences are amplified (e.g., by PCR), and finally the PCR products are hybridized to a set of probes immobilized on the chip.

Hybridization kinetics of nucleic acids is temperature dependent, and the specificity and efficiency depend on the hybridization temperature. Traditionally, the hybridization is performed in a water bath or on an automatic hybridization station.

The ultimate goal for biochip researchers is to develop a portable "lab-on-chip" system that could be used, for example, to detect environmental pathogens or other microbes at point of care with the minicyclers. However, the large and complex instruments required for DNA analysis greatly constrain such applications where portability is essential.

Chaotropic anions at high concentrations (e.g., trichloroacetate>trifluoroacetate>thiocyanate>perchlorate>iodide>bromide>acetate) can lower the T_m of dsDNA (5). The advantageous characteristics of chaotropic anions have been successfully applied in nucleic acid hybridization on nitrocellulose membranes, for example, using relatively dilute solutions of iodide and thiocyanate (6) and 3–6 M guanidine thiocyanate (7). We have studied the effect of 3 M guanidine thiocyanate on the temperature dependence of DNA chip-based hybridization.

A 244-bp-long conserved region of hepatitis C virus was used as the probe (8). A 1054-bp region of human lymphocyte antigen was used as the control probe (spotting control and position control). The probes (50 ng/ μ L) were prepared by PCR, purified, and immobilized onto a poly-L-lysine slide using the protocol released from Brown's Laboratory at Stanford (<http://cmgm.stanford.edu/pbrown/protocols/index.html>) (9). The target was prepared by PCR and la-

beled with CyTM5 by incorporating Cy5-dCTP during primer extension. Hybridization was performed in a humid chamber for 4 h. The hybridization buffer is prepared as follows: 3 M GuSCN, 2 $\times$ SSC, 0.5% SDS, and 5 $\times$ Denhardt's solution. Three different hybridization temperatures (15°C, 21°C, and 42°C) were explored. A control hybridization in 50% formamide buffer (50% formamide, 2 $\times$ SSC, 0.5% SDS, and 5 $\times$ Denhardt's solution) was performed in a humid chamber for 4 h at 42°C. Only the temperature points were tested in this experiment. After hybridization, all of the slides were washed with solution I (0.3 $\times$ SSC and 0.1% SDS) for 5 min at room temperature, followed by another wash in solution II (0.06 $\times$ SSC) for 5 min at room temperature. The slides were finally dried by centrifugation at 2000 rpm for 2 min.

The bound label on the microarray was imaged using a ScanArray[®] 4000 (GSI Lumonics, Wilmington, MA, USA) at 5 μ m resolution and 80% laser power and 80% photomultiplier tube

Figure 1. Hybridization with 2 M GuSCN under different temperatures. (a) Spotting pattern. ● - position control (Cy5-labeled human lymphocyte antigen PCR product); ○ - positive probe; ○ - blank control; ○ - negative probe (unlabeled human lymphocyte antigen PCR product). (b) 1%–50% formamide/42°C; 2–3 M GuSCN/15°C; 3–3 M GuSCN/21°C; 4–3 M GuSCN/42°C.

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Table 1. The Comparison of the Four Hybridization Conditions

a. 95% Confidence interval for mean of the signal intensity

1	4.29 ± 0.43
2	11.43 ± 2.16
3	9.42 ± 2.49
4	9.09 ± 1.49

b. Multiple comparisons of the four hybridization conditions

	1	2	3	4
1	-	0.002	0.017	0.001
2	0.002	-	0.618	0.256
3	0.017	0.618	-	1.000
4	0.001	0.256	1.000	-

Note: The significant value between each two conditions at a 95% confidence.

Table 2. 3 M GuSCN Buffer at 21°C (ImaGene, Method 2)

Positive Spots	Background	Negative Control	Positive/Negative
45.79	0.03	0.2	229
39.89	0.08	0.15	265.9
38.82	0.04	0.16	242.6

value unless otherwise stated. The images were analyzed using ImaGene™ (BioDiscovery, Marina del Rey, CA, USA). The fluorescence intensity was calculated as follows: mean (hybridization signal)/mean (signal of spotting control).

The results (Figure 1) indicate a significantly better hybridization performance in GuSCN (15°C, 21°C, and 42°C) compared with 50% formamide at 42°C ($P < 5\%$; Table 1). Hybridizations were performed under a wide range of temperature conditions from 15°C to 42°C, and there were no statistically significant differences across this range of hybridization temperatures ($P > 5\%$; Table 1). Additionally, there were almost no signals detected with the negative control (positive/negative > 200 ; Table 2), indicating that hybridization in GuSCN was highly sequence specific.

When the 3 M guanidine thio-

cyanate was included in the hybridization buffer, the melting temperature of hybrids and the optimum temperature of hybrid formation were greatly reduced. Using this hybridization buffer, the hybridization on a DNA chip could be accomplished over a wide range of temperature with the hybridization performance better than those in 50% formamide at 42°C.

Our experimental results reveal that concentrated GuSCN buffer is suitable for microarray-based hybridization reactions. This strategy eliminates the need for a complex temperature control unit and facilitates simplification and miniaturization of the process. Such a system may find use in oligonucleotide microarray analysis (10). Another advantage of this hybridization buffer is that it may be combined directly with the GuSCN

sample preparation method (11). When DNA and RNA are extracted from samples using the GuSCN method, they can be hybridized directly with the probes attached on the chip without prior nucleic acid purification (12). The present study provides an important methodological improvement that simplifies microarray-based hybridization and will simplify the construction of a sample-to-answer lab-on-chip system.

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