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SNP Detection Using Thermal Gradient DNA Chip

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1. Introduction

With the rapid increase of knowledge in the field of molecular biology, application of microtechnology to this field has become increasingly important for the parallell processing of biological information. We have made several approaches to realize intelligent and micro biochemical sensing systems using microfabrication technology[1].

In the fields of molecular biology and clinical research, DNA chips and DNA microarrays have been powerful tools for gene expression and genotypic analyses. Since the fundamental concept of DNA chips and DNA microarrays is parallel hybridization and washing between immobilized DNA probes and target DNAs in a sample on a solid substrate, differences in melting temperatures among DNA probes have to be taken into consideration to obtain reliable results, especially for DNA chips and DNA microarrays with oligonucleotide probes. Although several methods such as optimization of length and composition of DNA probes are reported to overcome this problem, it is, in general, difficult to find the optimum condition which is common to all DNA probes on the substrate. We have proposed a new DNA chip in which the temperature of each DNA probe can be controlled independently and set to an optimum value [4]. The concept of the new DNA chip is based on the large difference in thermal conductivity between silicon and silicon dioxide. Fundamental characteristics of the thermal gradient DNA chip are described in the present study.

2. Device structure of thermal gradient DNA chip

The structure of the thermal gradient DNA chip is shown in Fig. 1. The chip size is 26 mm × 26 mm. One hundred Si-islands are formed in the membrane of silicon dioxide and silicon nitride using an anisotropic etching technique of silicon. The size of Si-island is 500 μm×500 μm. A heater and a temperature sensor are fabricated in each Si-island. The heater is made of a diffusion layer and the temperature sensor is based on a pn junction. A cross-section of the chip is shown in Fig.1C. The height of the Si-islands is 250 μm and the thickness of the SiO_2 /Si_3N_4 membrane is about 4 μm. The fundamental concept of the new DNA chip is based on the large difference in heat conductivity between silicon and silicon dioxide. The heat conductivity of silicon crystal is 148 W/mK, while that of silicon dioxide is 10.4 W/mK.

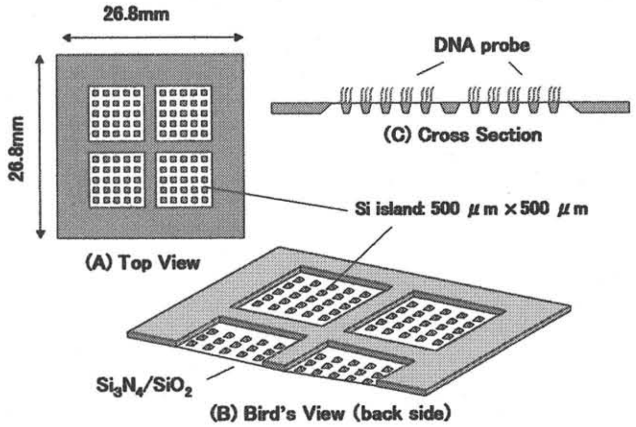


Fig. 1 Structure of Thermal Gradient DNA Chip

A photograph of the fabricated DNA chip is shown in Fig.2. The chip is mounted on a printed circuit board and wire-bonded. Hybridization and washing are carried out in the active area on the chip, which is surrounded by the white silicone rubber. The temperatures of 100 silicon islands can be controlled simultaneously using the fabricated control system, which is operated during the hybridization and washing processes.

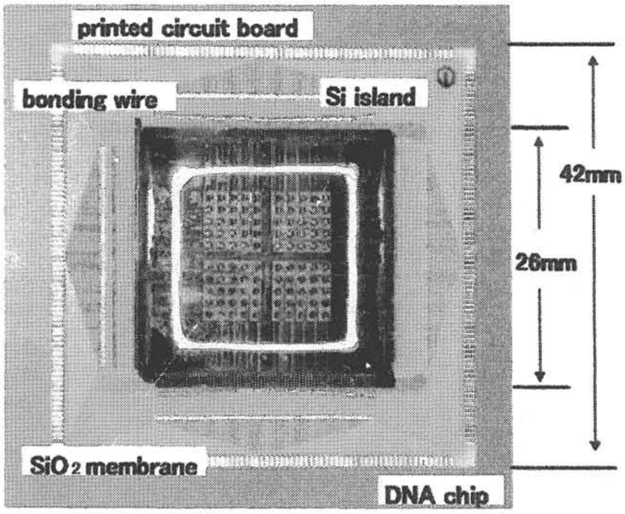


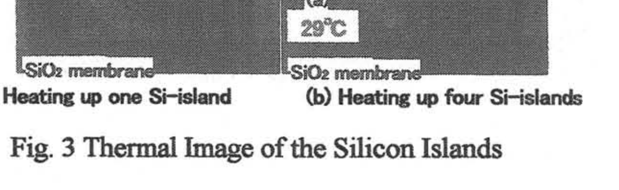
Fig. 2 Photograph of the Thermal Gradient DNA Chip

3. Fabrication process

The thermal gradient DNA chips were fabricated using n-type silicon having a resistivity of 8 – 12 Ωcm. The wafers were cleaned and thermally oxidized to 400 nm. A 50 nm-thick film of silicon nitride was then deposited on the silicon dioxide layer and p-wells were formed by ion implantation of BF^{2+} and subsequent diffusion process. After local oxidation of silicon(LOCOS), n-type regions were formed by ion implantation of As^{+} and subsequent diffusion process for heaters and temperature sensors. The n-type region for a heater is 1600 μm long and 20 μm wide. Sheet resistance of the n-type layer is 100 Ω/□. The Si-islands were formed using the anisotropic etching technique with a silicon nitride mask.

4. Thermal isolation characteristics

The thermal isolation characteristics of the chip is shown in Fig. 3. Only one island (a) was heated up to 45 ℃, while the other islands were unheated and left at 20 ℃. In this case, the temperatures of the neighboring islands were approximately 20 ℃ and there was little



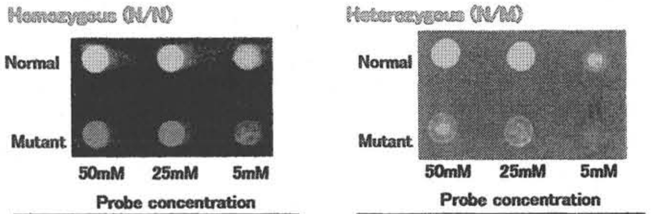
(a) Heating up one Si-island (b) Heating up four Si-islands

Fig. 3 Thermal Image of the Silicon Islands

influence on the neighboring Si-islands. When the four islands(b –e) were heated up to 45 ℃ simultaneously, the temperature of the central island (a), that was not heated, was found to increase up to 29 ℃.

5. SNP detection

A single base change in the Factor Ⅶ gene was tested using allele-specific oligonucleotide probes corresponding to normal and mutant alleles. Two different dye-tagged PCR targets with different genotypes(N/N, N/M) were hybridized and washed on the chip. As shown in Fig. 4, results of target hybridization demonstrate the distinction between two genotypes.



	50nM	25nM	5nM
Normal	101.2	67.6	44.8
Mutant	16.2	15.4	7.3
Ratio(N/M)	6.3	4.4	6.1

	50nM	25nM	5nM
Normal	59.7	51.9	21.9
Mutant	23.2	21.8	9.8
Ratio(N/M)	2.6	2.4	2.2

Signal intensity (Background subtracted)

Fig. 4 Hybridization results

6. Conclusion

We have developed a new DNA chip in which the temperature of each DNA probe can be controlled independently and set to an optimum value. We fabricated the prototype chip and evaluated the fundamental characteristics. The Si island can be thermally isolated from the neighboring ones effectively with the proposed structure. Using this new DNA chip, We can arrange the appropriate DNA probe on the chip depending on its melting temperature and hybridization between a DNA probe and a target DNA can be carried out in the optimum condition.

Acknowledgement

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Reference

1. T. Kajiyama, K. Murakawa and Y. Miyahara, Thermal Gradient DNA Chip, Proc. of Micro Total Analysis System 505(2000).