# Development of High-Throughput Pico-liter PCR System

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Polymerase chain reaction (PCR) has been applied to molecular biology, medicine and pharmaceutics. A demand for PCR in there fields is to analyze huge numbers of samples quickly and simultaneously. Miniaturized PCR devices can be integrated to high density and analyze many samples simultaneously and reduce the volume of the reagents (i.e., DNA polymerase). A highly integrated PCR device was fabricated by semiconductor microfabrication technology. The inner walls of the chambers were oxidized to be hydrophilic in order to retain the solution. The small volume viability of PCR was estimated by using the microchamber array. The minimum chamber size was 85-pL, and the density was 2500 chambers/$cm^2$. BSA was added in the PCR mixture in order to prevent absorption of contents, such as template and DNA polymerase. The amplification of a fragment of *gfp* gene was determined by technique based on energy transfer between two fluorescent dyes. When small numbers of molecules are reacted, a pico-liter chamber has higher efficiency in reacting the small numbers of templates than the usual µl or ml tube. Because the apparent concentration of template increases in such miniaturized volume for the same number of template in a chamber. The PCR was successfully carried out in the 85 pL microchamber from only three template molecules. To extract the PCR products, a porous Teflon membrane was used between the microchamber and a glass slip.

Silicon has excellent thermal conductivity, and the heat capacity is small in the volume-minimized solution. Hence we expect that it is able to transfer heat rapidly and therefore reduce the thermal cycle time. We suggest new thermal control method for rapid PCR. A thermal cycler for the microchamber array was developed with three heaters arrayed on an eccentric table. The microchamber array chip was repeatedly moved on these heaters to control the temperature. Rapid PCR was performed by using the microchamber array and the thermal cycler. Each heater was controlled thermostatically; 96˚C, 55˚C and 72˚C. Consequently, one cycle took for 40 sec. Compared with a commercial PCR instrument (Model 9600, PE Applied Biosystems-3hour), the total reaction time was 1/3.

The microchamber array was applied to a single cell PCR. Jurkat cells were used as a model cell. $1x10^4$ cells/ml Jurkat cell suspension was dropped on the microchamber array and the cells were separated in each microchamber according to Poisson distribution. The isolated cell rate was 48% in a whole cell. Proteinase K and Triton X-100 were necessary to lyse the cell. The single cell PCR was successfully carried out on the microchamber array.

In conclusion, a high-throughput PCR system was developed by using semiconductor microfabrication technology, and applied a single cell PCR. The single cell PCR on the microchamber array is able to identify and quantify the various kinds of cells and microorganisms in sample. This methodology was useful in wide area, such as medicine and environmental monitoring.