# HIGHLY FAST REAL-TIME PCR SYSTEM BASED ON RAPID THERMAL CYCLER AND 2-COLOR SCANNING OPTIC MODULE

**Wonseok Chung, Kak Namkoong, Chinsung Park, Wonjong Jung, Sunok Jung, Kyung-Ho Kim, Joon S. Shim, Kyu-Youn Hwang, Heekyun Lim, Joon-Ho Kim and Nam Huh**

*In Vitro Diagnostics Lab, Samsung Advanced Institute of Technology (SAIT), Samsung Electronics Co. Ltd., Korea*

## ABSTRACT

This paper reports a highly fast real-time polymerase chain reaction (PCR) system based on a rapid thermal cycler and a 2-color scanning optic module. Average heating and cooling rate of the rapid thermal cycler is 34.5 ◦C/s and -4.5 ◦C/s each and 0.5 nM of FAM and Texas Red dyes can be detected by 2-color scanning optic module. The *Staphylococcus aureus* (*S. aureus*) specific SA442 fragment was successfully amplified using the proposed real-time PCR system.

## KEYWORDS

Real-time PCR, Rapid thermal cycler, Scanning optic module

## INTRODUCTION

Real-time PCR has been widely used in biological research and diagnostics because of its advantages over end-point PCR, such as quantification, reduced analysis time, higher specificity and increased sensitivity over a wider dynamic range. Growing needs for real-time PCR technology result in a lot of real-time PCR instruments both in the research field and commercial market. We report a highly fast real-time PCR system based on a rapid thermal cycler and a 2-color scanning optic module, which allows shorter analysis time and higher throughput per test than those of other PCR platforms.

## EXPERIMENT

Figure1a shows the rapid thermal cycler and 2-color scanning optic module of real-time PCR system. The rapid thermal cycler for regulating temperature of a micro PCR chip consists of a silicon-based thermal plate and a fan for forced convective cooling. A thin-film platinum resistive heater and a resistance temperature detector were patterned on a silicon substrate by MEMS process (Figure 1b). Because silicon has a high thermal conductivity, temperature of the PCR chip can be easily elevated by heat generated from the platinum heater and drop down by air flow from the cooling fan. Micro PCR chips used in this study were made by anodic bonding between an etched silicon and a glass (Figure 1c) [1, 2]. The chip has 8 chambers whose volume is 1 μL each (Total 8 μL).

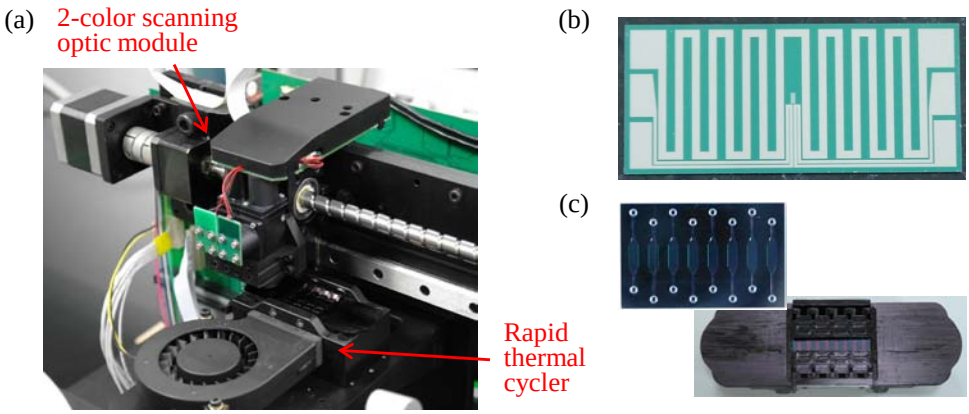


*Figure 1. Photos of highly fast real-time PCR system. (a) Rapid thermal cycler and 2-color scanning optic module, (b) Thermal plate (31 X 14 mm$^2$) and (c) Micro PCR chip (25 X 16 mm$^2$) in the package.*

## RESULTS AND DISCUSSION

In order to evaluate thermal cycler's performance, the temperature of a silicon chip which has the same size as the PCR chip was measured during thermal cycling between 55◦C (5 sec) and 95 ◦C (1 sec ). Figure 2 shows that average heating rate of thermal cycler in the range of 60~90℃ is 34.5 ◦C/s and cooling rate is -4.5 ◦C/s and these ramping rates are considerably higher than those of most commercial PCR machines.

The 2-color optics can measure fluorescence signal in two channels without crosstalk. Blue and orange LEDs are used for exciting fluorescent dyes and optical filters are adequately designed. As shown in Figure 3, FAM and Texas Red dyes can be detected as low as 0.5 nM. During thermal cycling, the 2-color optics scans the PCR chip at the appropriate period of each cycle (Figure 4a) and fluorescence signals from the each chamber region are post-processed. PCR curve is drawn from the post-processed data (Figure 4c) and threshold cycle (Ct) can be determined from the PCR curve. PCR reaction in all chambers can be monitored in a very short time (~0.4 sec) because average scanning speed is 15 cm/s.



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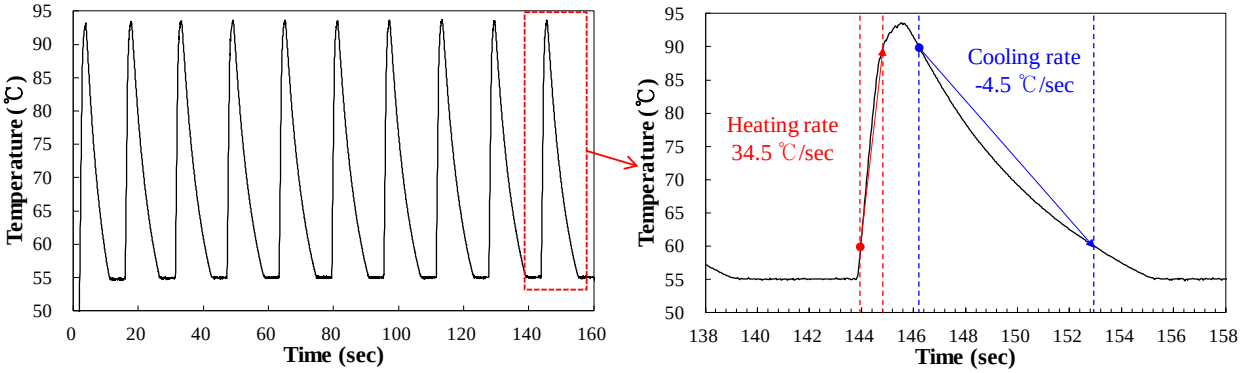


*Figure 2. Measured temperature profile of silicon chip during thermal cycling*

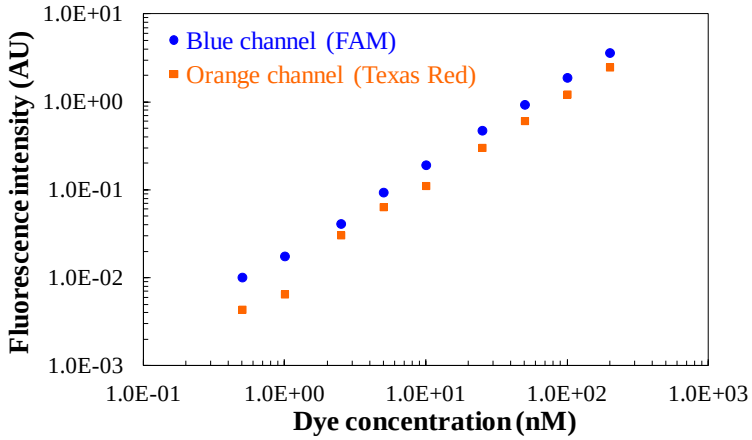


*Figure 3. Fluorescence signal measurement using 2-color scanning optic module.*

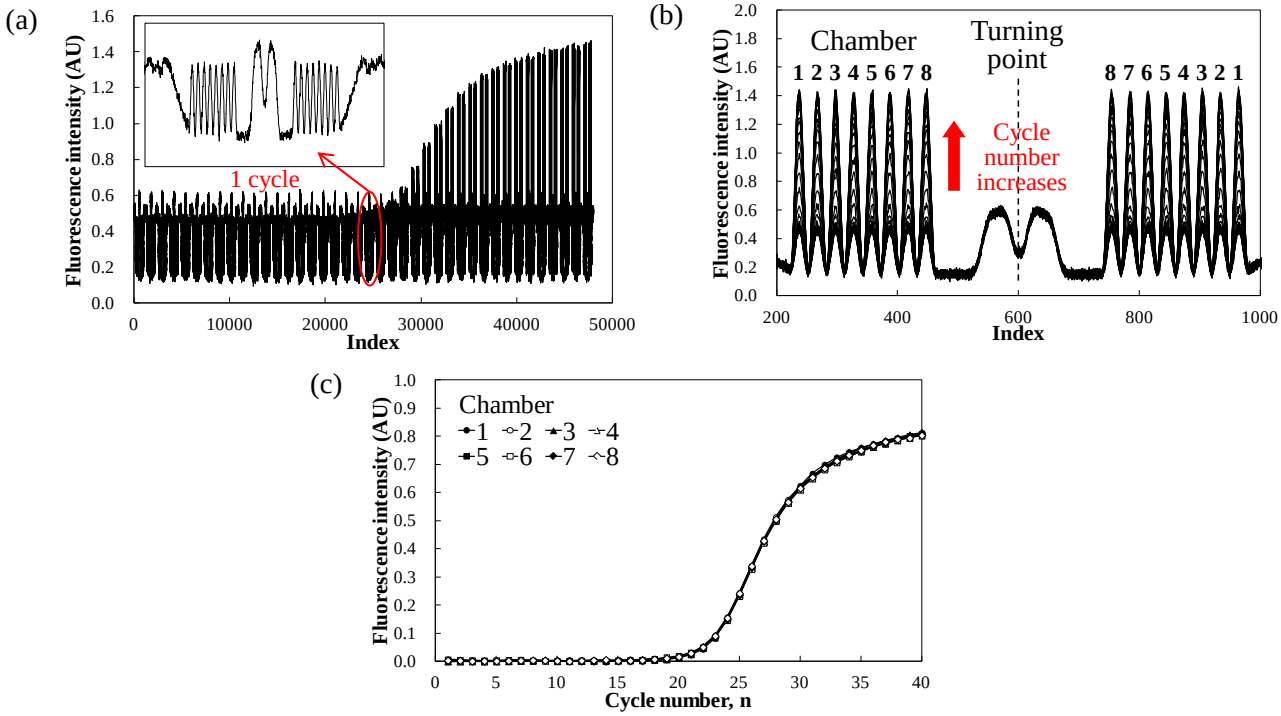


*Figure 4. Fluorescence signal from scanning optic module. (a) All scanned data during 40 thermal cycles, (b) Stacked data along scanning position and (c) PCR curves for each 8 chamber (%CV of Ct: 0.73%).*

Fragment of the SA442 region within *S. aureus* genome was amplified in order to evaluate the performance of real-time PCR system. The size of PCR amplicons was designed to be 76 base pairs. Five different concentrations ($10^4$~$10^0$ copies/μL) of templates were amplified and detected in blue channel and one concentration ($10^4$ copies/μL) in orange channel. TaqMan® probes were used for real-time detection. Forty cycles of denaturation at 95℃ for 1

sec and annealing-extension step at 60 ◦C for 5 sec were repeated in less than 10 minutes. Scanning for detecting fluorescence signal started at 3.5 sec of each extension step. As shown in Figure 5, real-time PCR was successfully performed in both channels.

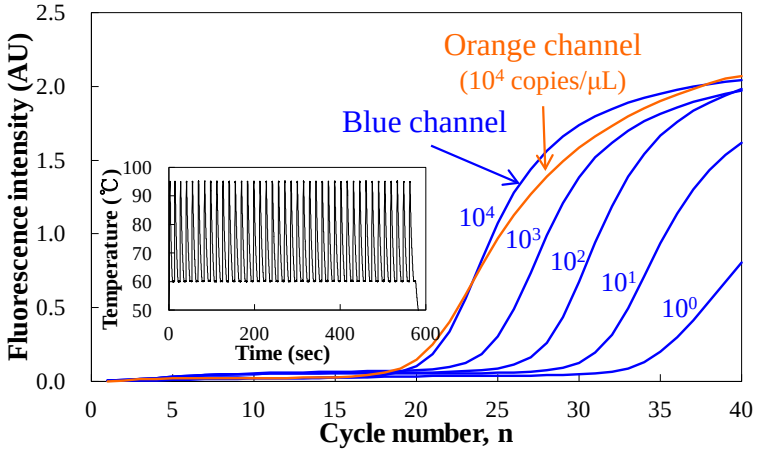


*Figure 5. Result of real-time PCR in less than 10 minutes (Inserted graph: temperature sensor signal on thermal plate during thermal cycling).*

## REFERENCES


[1] Y.-K. Cho, J. Kim, Y. Lee, Y.-A. Kim, K. Namkoong, H. Lim, K. W. Oh, S. Kim, J. Han, C. Park, Y. E. Pak, C.-S. Ki, J. R. Choi, H.-K. Myeong and C. Ko, *Clinical evaluation of micro-scale chip-based PCR system for rapid detection of hepatitis B virus*, Biosensors and Bioelectronics, 21, pp.2161-2169, (2006).

[2] K.W. Oh, C. Park, K. Namkoong, J. Kim, K.-S. Ock, S. Kim, Y.-A. Kim, Y.-K. Cho and C. Ko, *World-to-chip microfluidic interface with built-in valves for multichamber chip-based PCR assays*, Lab on a chip, 5, pp.845-850, (2005).


## CONTACT


Joon-Ho Kim 82-31-280-6939 or mythos.kim@samsung.com