Temperature overshoot improves speed of PCR

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The Spartan DX™ performs real-time PCR using fixed temperature heat blocks. Compared to Peltier-based thermal cyclers, run times may be twice as fast because no time is lost in ramping heat block temperatures. Here we show that run times can be improved even further using a simple temperature overshoot protocol (patent pending).

Introduction

Within its permissive temperature range standard *Taq* DNA polymerase has a DNA elongation rate of 50 bp/s (Ref 1). Therefore, to successfully synthesize an amplicon of 100-250 bp, it should only be necessary for the reaction mixture to remain within this temperature range for a total of 2-5 s. In fact, some studies have shown that successful PCR can occur when the reaction mixture remains at the optimal denaturation/annealing temperatures for as little as 1 s (Ref 2).

Traditionally, 94-96°C is the maximum temperature used for the denaturation step of PCR. This is because temperatures higher than 96°C lead to heat inactivation of the *Taq* polymerase and subsequent failure of the reaction (Ref 3).

If the temperature of a heat block was set much higher than 96°C, e.g. 105°C, it might still be possible to perform productive PCR if the reaction tube was moved out of contact with the heat block once the internal fluid temperature reached a maximum of 96°C. In this way, the heat block could be set at 105°C, but the temperature of the reaction mixture would never be allowed to reach 105°C. The advantage of such a temperature overshoot method would be rapid heating of the reaction mixture because of the larger heating gradient.

The Spartan DX is a real-time DNA analyzer that uses fixed-temperature heat blocks to perform real-time PCR. Compared to Peltier-based thermal cyclers, run times may be twice as fast because no time is lost in ramping block temperature (Ref 4).

The purpose of this study was to determine if a temperature overshoot method would facilitate efficient PCR and decrease run times.

Materials and Methods

DNA extraction

DNA was isolated from clinical isolates of *Staphylococcus aureus*. For each isolate, 4-5 medium-sized bacterial colonies were re-suspended in 100 µl of lysis buffer (50 mM Tris-HCl, 50 mM NaCl, 5 mM EDTA, pH 8) with 2 µl of 1 mg/ml lysostaphin (Sigma-Aldrich, Cat No. l7386). The samples were incubated at 37°C for 30 min. Following this incubation, 5 µl of 20 mg/ml Proteinase K (Sigma-Aldrich, Cat. No. p2308) was added to the mixture and the tubes were shaken at 50°C for 1 h. The tubes were then incubated at 100°C for 10 min to inactivate the Proteinase K. Extracts were diluted to a concentration of 750 ng/µl, aliquoted, and stored at -20°C.

Determination of temperature overshoot protocol

A thermocouple (Omega, Part# 5SRTC-TT-T-40-72) was used to measure the reaction liquid temperature. Specifically, a thin-wall 0.2 ml polypropylene tube (Axygen, Cat. No. PCR 02C) was altered in the following manner: a hole of 1 mm in diameter was drilled into the top of the tube, the thermocouple was threaded through the hole, and the tip of the thermocouple was positioned 1-2 mm from the bottom and equidistant from the sides. The tube was then filled with 20 µl of distilled water, and topped with 15 µl of mineral oil. Temperature was recorded every second for 500 s using a data logging thermometer (Fluke, 54II).

The temperatures of the blocks were set at 105°C and 50°C. Using the thermocouple setup, it was determined that dwell times of 13 s and 18 s at 105°C and 50°C respectively enabled the liquid temperature to reach 88°C and 64°C, respectively (Table 3).

Component	Final Amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	2.5 mM
dNTP mix (Invitrogen)	0.125 mM
Taq DNA polymerase (Invitrogen)	1 U
SYBR Green I (Invitrogen)	0.5 X
PCR primers (Biosearch Technologies)	0.5 µM
Template DNA	1.5 µg
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components of amplification mixture.

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Program	Step	Temp	Time	Cycles
1	Initial denaturation	94°C	30 s	1
	Denaturation	94°C	30 s	35
	Annealing/extension	58°C	30 s	35
2	Initial denaturation	105°C	20 s	1
	Denaturation	105°C	11 s	35
	Annealing/extension	50°C	16 s	35

Table 2. Cycling parameters.

Program	High Temp	Low Temp
1	88°C	63°C
2	88°C	64°C

Table 3. Maximum and minimum temperatures (Temp) achieved with and without temperature overshoot program.

Real-time PCR

Oligonucleotide primers were designed against a conserved region of the bacterial Sa442 DNA fragment (Ref 5). The forward primer sequence was 5'-tcg gta cac gat att ctt cac-3'and the reverse primer was 5'-act ctc gta tga cca gct tc-3'. The expected amplicon size was 179 bp (Ref 5).

Components of the real-time PCR amplification mixture are listed in Table 1. SYBR Green I dye (Invitrogen, Cat. No. 57563) was used for fluorescent detection. Samples were loaded into 0.2 ml thin-wall, flat cap PCR tubes (Axygen, Cat. No. PCR 02C), and topped with 15 µl of mineral oil (Biotools, Cat. No. 20.032) to prevent evaporation. Real-time PCR was performed using the Spartan DX device. Table 2 shows the cycling parameters for two-temperature programs which combine the primer annealing and extension steps. All reactions were performed in triplicate.

DNA analysis

Fluorescence data from each program were downloaded from the Spartan DX to a computer and graphed using Microsoft Excel®. In addition, real-time PCR results were confirmed by agarose gel electrophoresis using 10 µl of the reaction mixtures.

Results

Real-time results and threshold cycle (Ct) values were equivalent between temperature overshoot and non-temperature overshoot programs (Fig 1 & 2). The total run time was reduced from 29 min (Program 1) to 21 min (Program 2). Gel electrophoresis confirmed amplification of the expected 179 bp fragment for all samples examined (Fig 3).

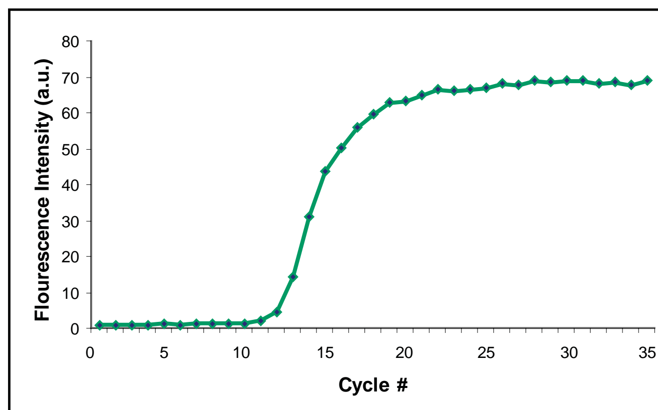

Figure 1. Real-time PCR result without temperature overshoot (Program 1).

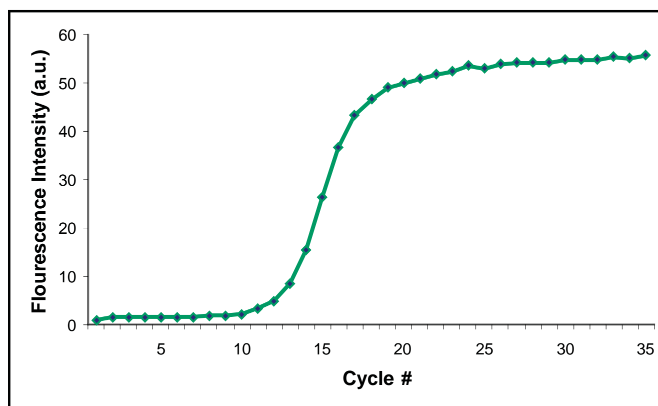

Figure 2. Real-time PCR result with temperature overshoot (Program 2).

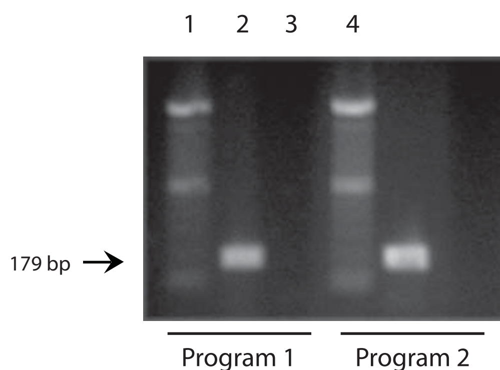

Figure 3. Gel electrophoresis of PCR products. The expected band size of 179 bp was detected in lanes 2 and 5. Lanes 1 and 4 contain a 100 bp DNA ladder and lanes 3 and 6 are negative controls (no DNA).

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Discussion and Conclusions

The results of this study demonstrate that a simple temperature overshoot method decreased reaction time by nearly 30% with no effect on the PCR quality.

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