SYBR® Green I concentrations for real-time PCR

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The Spartan DX™ performs best with SYBR Green I concentrations ranging from 0.2 - 0.7 X. The optimal concentration is 0.4 X.

Introduction

SYBR Green I is a DNA-intercalating dye that is widely used for real-time PCR applications (Ref 1). It binds within the minor groove of double-stranded DNA and emits a fluorescent signal with a peak at 525 nm. Since SYBR Green I preferentially binds to double-stranded and not single-stranded DNA, it can be used to monitor the increase in double-stranded products during PCR.

There are advantages and disadvantages to using SYBR Green I for real-time PCR detection. The advantages include high detection sensitivity and low reaction cost compared to fluorescent probes. The disadvantage is that it binds to any double-stranded DNA, including non-specific primer-dimers. In addition, SYBR Green I is known to inhibit PCR at higher concentrations (Ref 2). Conversely, too low a concentration of SYBR Green I may make it difficult to detect the increase in PCR products.

The purpose of this study was to determine a useful concentration range for SYBR Green I for the Spartan DX personal DNA analyzer.

Materials and Methods

DNA extraction

DNA was extracted 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 incubation, 5 µl of 20 mg/µl Proteinase K (Sigma-Aldrich, Cat. No. p2308) was added to the mixture and 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 adjusted to 750 ng/µl, and stored at -20°C.

Real-time PCR

Oligonucleotide primers were designed against a conserved region of the bacterial *16S* gene. The forward primer sequence was 5'-cga aag cgt ggg gat caa ac-3' and the reverse primer was 5'-ccc agg cgg agt gct taa tg -3'. The primers were designed with Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The expected amplicon size was 125 bp.

Components of the real-time PCR amplification mixture are listed in Table 1. A 10,000 X stock of SYBR Green I (Invitrogen, Cat. No. 57563) was serially diluted so that final reaction concentrations ranged from 0.1 to 1.2 X. 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 a two-temperature program, which combines the PCR annealing and extension steps. All reactions were performed in triplicate.

DNA analysis

Fluorescence data from the real-time runs was downloaded from the Spartan DX to a computer and graphed using Microsoft Excel®. Results were confirmed by gel electrophoresis using 10 µl of the amplification products.

Results

Threshold cycles (Ct) for real-time PCR ranged from 11 to 12 with SYBR Green I concentrations of 0.2 to 0.7 X (Fig 1). Concentrations greater than 0.7 X resulted in a delay of the Ct values from 17 to 31 (Fig 2). Concentrations less than 0.2 X were difficult to distinguish from baseline fluorescence. Gel electrophoresis confirmed amplification of the expected

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)	0.1 - 1.2 X
PCR primers (Sigma-Aldrich)	0.5 µM
Template DNA	1.5 µg
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components of amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	93.6°C	30 s	1
Denaturation	93.6°C	30 s	30
Annealing/extension	56.9°C	30 s	30

Table 2. Cycling parameters.

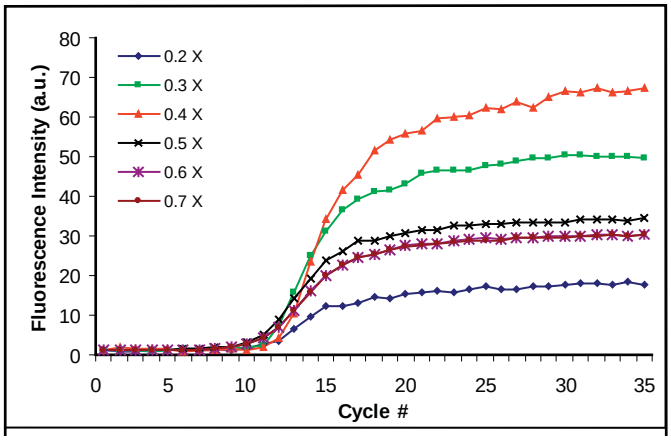

Figure 1. Real-time PCR results with SYBR Green I concentration ranging from 0.2 to 0.7 X.

125 bp fragment for samples containing 0.1 X to 0.8 X SYBR Green I (data not shown). We also observed variations in SYBR Green I fluorescence depending on the lot used.

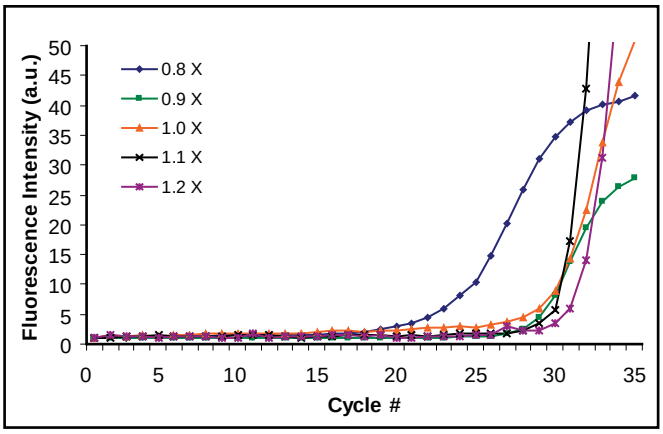

Figure 2. Real-time PCR results for SYBR Green I concentrations ranging from 0.8 to 1.2 X (delayed Ct).

Discussion and Conclusions

We observed that concentrations of SYBR Green I above 0.7 X were inhibitory to PCR as evidenced by the delay in Ct value. This was expected based on previous reports in the literature (Ref 3). Concentrations less than 0.2 X were too low to be detectable above baseline fluorescence. Therefore, a SYBR Green I concentration of 0.2 to 0.7 X appears to be a workable range for the Spartan DX. To avoid problems stemming from variations between lots, we recommend using 0.4 X SYBR Green I for most real-time PCR applications.

References

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2. Epsy MJ, et al (2006). Real-Time PCR in clinical microbiology: applications for routine laboratory testing. *Clinical Microbiology Reviews.* 19(1):165-256.
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