TaqMan® chemistry is compatible with Spartan DX™

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The Spartan DX™ is able to perform real-time PCR with TaqMan® probes. Compared to SYBR Green® I, TaqMan probes increase the specificity of real-time PCR.

Introduction

TaqMan probes are oligonucleotides with reporter fluorophores at one end and quenchers at the other end (Ref 1). When a probe is free in solution, the fluorescence emitted from the fluorophore is decreased due to its proximity to the quencher. During PCR extension, the fluorophore and quencher are separated during amplification by the 5′→3′ exonuclease activity of the DNA polymerase resulting in an increase in fluorescence.

A major advantage of TaqMan probes is that fluorescence only increases if there is specific binding between the probe and template. In contrast, a DNA-intercalating dye such as SYBR Green I detects any double-stranded DNA, including both specific and non-specific PCR products.

The purpose of this study was to demonstrate the compatibility of TaqMan probes for amplicon detection using the Spartan DX.

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 750 ng/µl and stored at -20°C.

Real-time PCR

Oligonucleotide primers were designed against the Sa442 DNA fragment, which is specific to *Staphylococcus aureus*. 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′, producing a 179 bp amplicon (Ref 2).

Two fluorophore-quencher probes were obtained from Biosearch Technologies (www.biosearchtech.com). The first probe combined a CAL Fluor® GOLD 540 fluorophore with a BHQ®-1 quencher, and the second combined a FAM fluorophore with a BHQ-1 quencher. For both probes, the fluorophore was attached to the 5′ end and quencher to the 3′ end of the oligonucleotide. The sequence of both probes was 5′-tac tga aat ctc att acg ttg cat cgg aaa ca-3′.

Amplifications with the two TaqMan probes and with SYBR Green I were performed on the Spartan DX using 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. Components of the PCR amplification mixture are listed in Table 1. Two-temperature cycling parameters for SYBR Green I (Table 2) and TaqMan (Table 3) are listed below.

DNA analysis

Component	Final amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	2.5 mM
dNTP mix (Invitrogen)	1.25 mM
Taq DNA polymerase (Biotools)	1 U
SYBR Green (Invitrogen)	0.5 X
Probes (Biosearch Technologies, Inc.)	0.2 µM
PCR primers (Biosearch Technologies, Inc.)	0.5 µM
Template DNA	1.5 µg
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95.5°C	30 s	1
Denaturation	95.5°C	27 s	35
Annealing/extension	52.3°C	17s	35

Table 2. Cycling parameters for SYBR Green I.

Step	Temperature	Time	Cycles
Initial denaturation	95.5°C	1 s	1
Denaturation	95.5°C	45 s	35
Annealing/extension	52.3°C	45 s	35

Table 3. Cycling parameters for both TaqMan probes and SYBR Green I.

Fluorescence values from each cycle 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 analysis using 10 µl of the amplification products.

Results

The primer sets matched to the GOLD and FAM probes were first tested with SYBR Green I using the program in Table 2 (data not shown). The same program was also used for TaqMan probes and yielded a single amplicon of the correct size by agarose gel electrophoresis, but no detectable real-time fluorescence increase (data not shown). When the dwell time at the lower temperature was increased to 45 s, as seen in Table 3, both probes and SYBR Green I yielded PCR products with threshold cycles (Ct) ranging from 11 (SYBR Green I) to 17 (Fluor Gold) (Fig 1-3).

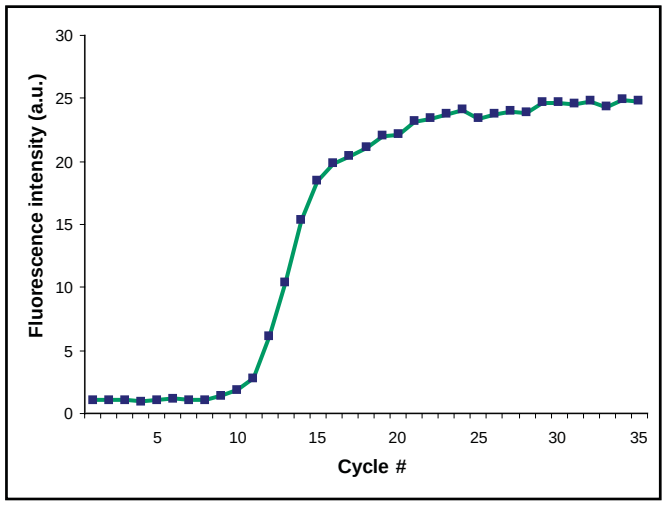

Figure 1. Real-time PCR result with 0.5 X SYBR Green I

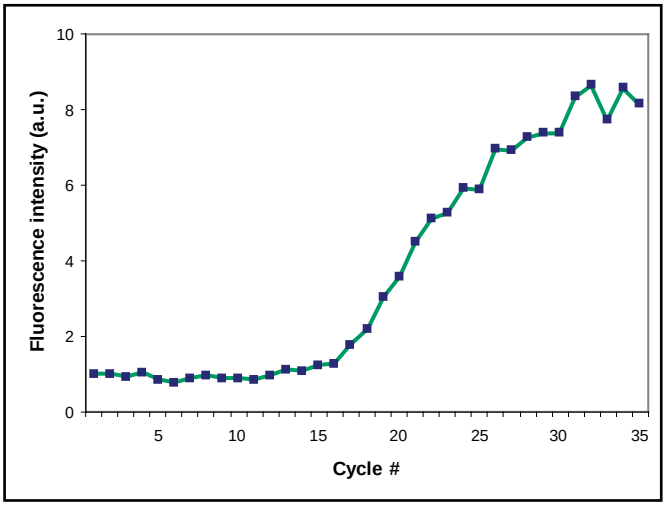

Figure 2. Real-time PCR result with 5′-CAL Fluor Gold probe.

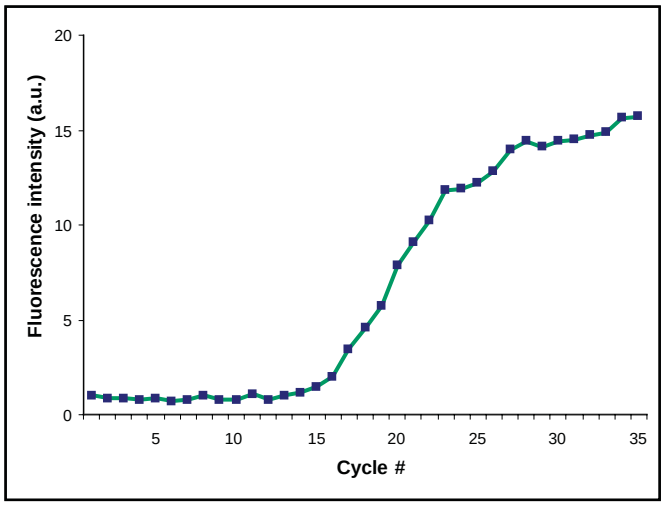

Figure 3. Real-time PCR result with 0.1 µM 5′-FAM probe.

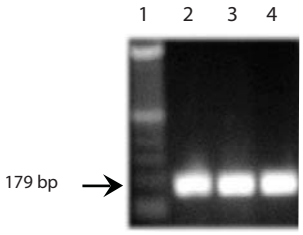

Figure 4. Gel electrophoresis of PCR products. Lane 1 contains 100 bp DNA ladder. Lanes 2-4 contain resultant PCR products with a specific amplicon of 179 bp, where Lane 2 is the product produced using SYBR Green I, Lane 3 using 5′-FAM probe and lane 4 using 5′-CAL Fluor Gold 540 probe.

Discussion and Conclusions

The results demonstrate that the Spartan DX is able to perform real-time PCR using TaqMan probes. For the TaqMan probes, it was necessary to extend the duration of the annealing/extension step in order to give the probes sufficient time to anneal to the amplicon. This problem can be circumvented by using other probe chemistries (such as Scorpion® probes, Molecular Beacons®, MGB® probes, or Lionprobes®) that by their nature either increase the binding kinetics of the probe to the amplicon or eliminate the need for a separate probe.

We also observed that Ct values with TaqMan probes were

delayed compared to SYBR Green I. This was likely due to the high baseline fluorescence with TaqMan probes and reflected the fact that the TaqMan reactions had to achieve a higher fluorescence level before the threshold cycle was detectable. Again, this problem can be overcome by using any of the othe probe chemistries described above since they are better at quenching the fluorescence of the fluorophore.

In summary, the Spartan DX is compatible with TaqMan probes.

References

1. Holland PM et al. (1991). Detection of specific Polymerase Chain Reaction product by utilizing the 5′ to 3′ exonuclease activity of *Thermus aquaticus* DNA Polymerase. *Proceedings of the National Academy of Sciences*. 88: 7276-7280.

2. Sabet Negar Shafiei et al. (2006). Simultaneous species identification and detection of methicillin resistance in *staphylococci* using triplex real-time PCR assay. *Diagnostic Microbiology and Infectious Disease*. 56: 13-18.