SYBR® Green master mix kits work with Spartan DX™

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The Spartan DX™ personal DNA analyzer is compatible with commercially available SYBR Green I master mix kits for Real-time PCR.

Introduction

Real-time PCR has substantially increased the speed by which genetic testing may be accomplished (Ref 1). To develop a real-time PCR assay, scientists can mix and match reagents from different manufacturers in a homebrew fashion. Alternatively, they may use off-the-shelf master mix kits. Master mixes have the advantage of simplifying and standardizing reagents, thereby reducing the likelihood of pipetting errors. Also, various additives in commercial master mix kits can reduce the need for optimization of PCR primers and probes.

The purpose of this study was to determine the compatibility of commercially available real-time PCR master mix kits with 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. Samples at a concentration of 750 ng/µl were stored at 4°C.

Real-time PCR

Oligonucleotide primers were designed against a conserved region of the bacterial *16S rRNA* 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.

The following master mix kits were tested: TAQurate™ GREEN Real-Time PCR MasterMix (EPICENTRE Cat. No. TM049096), iQ SYBR Green Supermix (Bio-Rad, Cat. No. 170-8880), Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, Cat. No. 11733-038), QuantiMix Easy SYG (Biotools, Cat. No. 10.607), FastStart SYBR Green Master (Roche, Cat. No. 04 673 484 001) and SYBR® GreenER™ qPCR SuperMix Universal (Invitrogen, Cat. No. 11762-100). A homebrew mix was also tested using the components in Table 1 together with final concentrations of 1X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen), 2.5 mM $MgCl_2$ (Invitrogen), 0.125 mM dNTP mix (Invitrogen, Cat. No. 10297018), and 1.5 U Taq DNA polymerase (Biotools, Cat. No. BT10048).

Amplifications 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. . Cycling parameters are listed in Table 2. All reactions were performed in triplicate.

DNA analysis

Fluorescence values 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 amplification products.

Results

All of the SYBR Green reaction mixes produce successful results (Table 3), despite the fact that all reactions were set up at room temperature. Hot Start kits produced equivalent results to their non-Hot-Start counterparts with no

Component	Final Concentration
16S primers (Biosearch Technologies)	0.5 µM
Template DNA	1.5 µg
Total reaction volume	**20 µl**

Table 1. Common components of amplification mixtures.

Step	Temperature	Time	Cycles
Initial denaturation	93.6°C	150 s*	1
Denaturation	93.6°C	30 s	35
Annealing/extension	56.9°C	30 s	35

**For the Roche and Invitrogen kits, the initial denaturation time was increased to 570 s.*

Table 2. Cycling parameters.

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differences in non-specific amplification products or primer-dimers as observed by agarose gel electrophoresis.

Discussion and Conclusions

The results showed that all of the commercial master mix kits were compatible with the Spartan DX™. The hot start method had no observable benefit for PCR efficiency, non-specific amplification products, or formation of primer-dimers with the primers used in this study. It was noted that freeze-thawing of master mixes containing SYBR Green resulted in degradation of the SYBR Green and decreased fluorescence. To avoid this problem, we recommend aliquoting master mixes after the first freeze-thaw, in appropriate volume sizes for single use.

In summary, the Spartan DX™ is compatible with real-time PCR master mix kits from a variety of manufacturers.

Master mix kit	Hot Start	Ct
Homebrew mixture	No	11.7 ± 1.2
TAQurate™ GREEN Real-Time PCR MasterMix (EPICENTRE)	No	10.7 ± 1.2
iQ SYBR Green Supermix (Bio-Rad)	No	10.7 ± 1.5
Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen)	2 min	12.7 ± 0.6
QuantiMix Easy SYG (Biotools)	No	14.0 ± 1.0
FastStart SYBR Green Master (Roche)	10 min	14.0 ± 1.0
SYBR® GreenER™ qPCR SuperMix Universal (Invitrogen)	10 min	15.3 ± 0.6

Table 3. Master mix kits and threshold cycles (Ct).

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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