illustra™ PCR Beads for convenient real-time PCR

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The illustra PCR beads are pre-mixed, pre-dispensed, single-dose reactions that enable convenient set up of real-time PCR amplifications on the Spartan DX™

Introduction

The illustra Ready-To-Go™ (RTG) PCR beads (GE Life Sciences) contain lyophilized reagents for real-time PCR and are stable at ambient temperatures. Each reaction uses a single bead and the user simply adds PCR primers, template DNA, water, and fluorescent probes or dyes. Advantages include decreased reaction preparation time, decreased pipetting errors, and standardization of reagents.

The purpose of this study was to determine the compatibility of the illustra beads for real-time PCR on 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 were adjusted to a concentration of 750 ng/µl and stored at -20°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 illustra RTG beads come in a Hot-Start (Cat. No. 28-9006-53) and non-Hot-Start format (Cat. No. 27-9559-01). Reaction mixtures were assembled according to the manufacturer's instructions (Table 1). $MgCl_2$ concentrations were adjusted to 2.5 mM. Cycling parameters are listed in Table 2. The hot-start beads were pre-incubated for an additional 2 min to activate the Hot-Start enzyme. Reactions 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. All reactions were replicated 3 times.

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

The Hot-Start and non-Hot-Start illustra RTG beads bothproduced successful real-time PCR results. For both types of beads, the average threshold cycle (Ct) values were equivalent (Table 3). There were no non-specific amplification products as analyzed by gel electrophoresis for either type of bead.

Discussion and Conclusions

Component	Final Concentration
illustra RTG beads	1 bead
$MgCl_2$ (Invitrogen)	2.5 mM
SYBR® Green I (Invitrogen)	0.5 X
PCR primers (Sigma-Aldrich)	0.5 µM each
Template DNA	1.5 µg
Sterile water	
Total reaction volume	**25 µl**

Table 1. Components of reaction mixtures.

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

* The Hot Start kit was pre-incubated for an extra 2 min.

Table 2. Cycling parameters.

Master Mix Kit	Hot Start	Ct
illustra PCR bead (Hot-Start)	2 min	12 ± 1.0
illustra PCR bead (non-Hot-Start)	No	11.6 ± 0.6

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

Both the Hot-Start and non-Hot-Start illustra PCR beads generated successful real-time PCR results with SYBR Green I and primers for the *16S rRNA gene*. To reduce run times, non-Hot-Start beads can be used, as no difference in specificity of results with the Hot-Start beads was detected.

In summary, illustra beads may be used for real-time PCR with the Spartan DX for more convenient preparation of reaction mixtures.