Fluorophores compatible with the Spartan DX™

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Green- and yellow-emitting fluorophores are compatible with the Spartan DX™. Examples include FAM, CAL Fluor® Gold 540, and CAL Fluor Orange 560.

Introduction

There are a variety of commercially-available fluorophores that may be used with real-time PCR chemistries such as TaqMan® (Applied Biosystems), Molecular Beacons (PHRI), and Scorpions™ (DxS). Green emitters have emission wavelengths from 500 to 549 nm and include fluorophores such as FAM, JOE, and TET™. Yellow emitters have emission wavelengths from 550 to 584 nm and include fluorophores such as TAMRA, CAL Fluor® Gold 540, HEX™, and VIC. The optical detection system in the Spartan DX is designed to detect fluorophores with excitation wavelengths from 400 to 500 nm and emission wavelengths from 500 to 700 nm. The purpose of this study was to evaluate the compatibility of fluorophores with different excitation/emission wavelengths with the Spartan DX.

Materials and Methods

DNA extraction

DNA was isolated from human whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Cat. No. 51104) according to the manufacturer's instructions. The samples were then boiled for 2 min and stored at 4°C.

Real-time PCR

Oligonucleotide primers were designed against a conserved region of the *human muscarinic acetylcholine receptor subtype M3 gene (CHRM3)* gene using online software from Biosearch Technologies (www.qpcrdesign.com). The forward primer sequence was 5'-ttg ggt cat ctc ctt tg-3', and the reverse primer was 5'-gca cag ttc tct ttc ca-3'. The expected amplicon size was 74 base pairs.

Six TaqMan fluorophore-quencher probe combinations were obtained from Biosearch (Table 1). The probe sequence was 5'-tcc ttt ggg ctc ctg cca tct-3' and consisted of a fluorophore at the 5' end and a Black Hole Quencher® (BHQ-1) at the 3' end.

TaqMan reactions were performed on the Spartan DX using 0.2 ml thin-wall, flat cap PCR tubes (VWR Cat. No. 53550-106). Components of the real-time PCR amplification mixture are listed in Table 2. Table 3 shows the cycling parameters for a two-temperature program which combines the primer annealing and extension steps.

Fluorophores	Excitation (nm)	Emission (nm)
FAM	492-494	517-522
CAL® Fluor Gold 540	522	544
CAL® Fluor Orange 560	538	559
Quasar® 570	548	566
CAL® Fluor Red 590	569	591
CAL® Fluor Red 610	590	610

Table 1. Fluorophore excitation and emission wavelengths.

Component	Final amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	5.75 mM
dNTP mix (Invitrogen)	0.2 mM
Platinum TAQ Polymerase (Invitrogen)	1 U
TaqMan Probes (Biosearch)	0.1 µM
PCR primers (Biosearch)	0.3 µM each
Template DNA	2 µl
Sterile water	
Total reaction volume	**20 µl**

Table 2. Components of real-time PCR mixture.

Step	Temperature	Time	Cycles
Initial denaturation	93.5°C	150 s	1
Denaturation	93.5°C	30 s	40
Annealing/extension	58°C	55 s	40

Table 3. Cycling parameters.

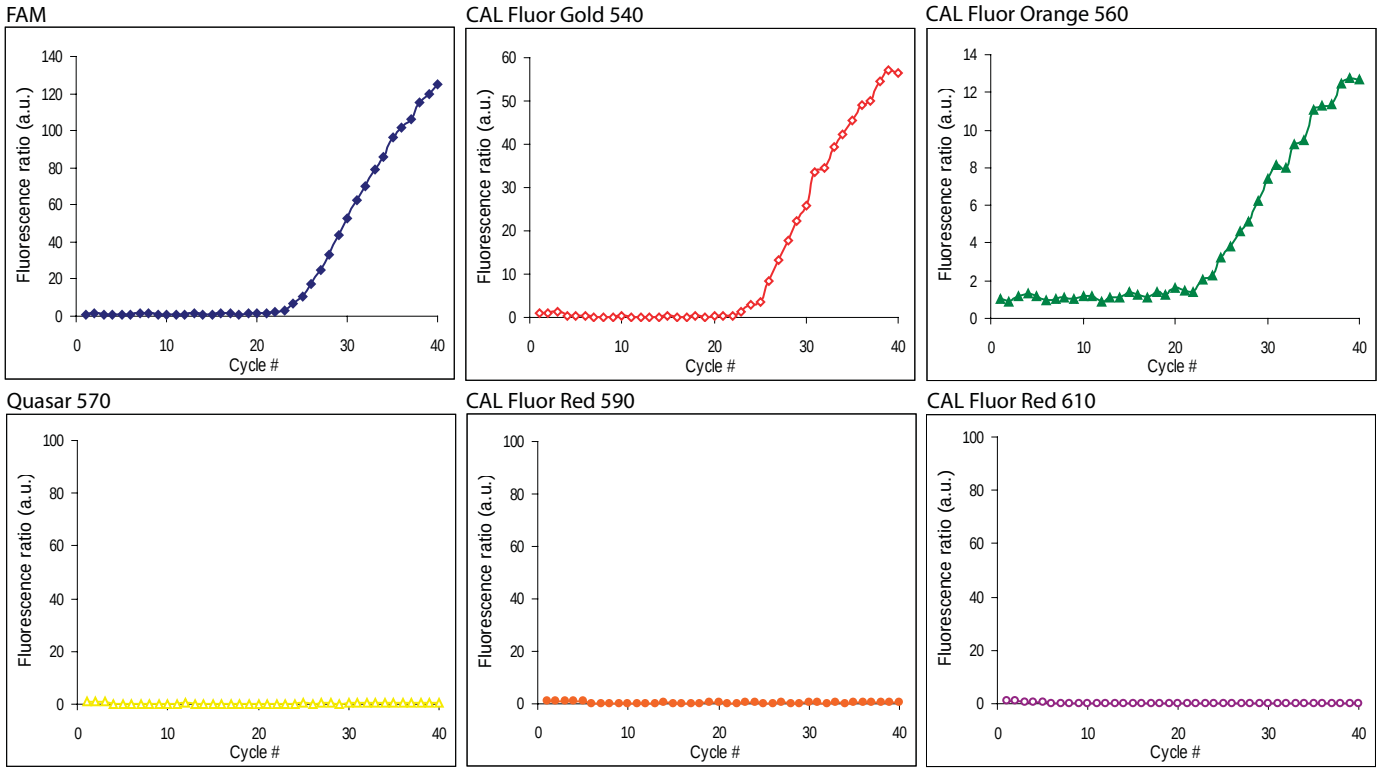

Figure 1. Real-time PCR results with six different fluorophores (a.u. = arbitrary units).

DNA analysis

Fluorescence values were downloaded from the Spartan DX to a computer and graphed using Microsoft Excel®. PCR results were confirmed by agarose gel electrophoresis using 5 µl of the amplification products.

Results

Gel electrophoresis revealed that all reactions yielded a single amplicon of the correct size. Real-time PCR graphs showed that samples with FAM, CAL Fluor Gold 540, and CAL Fluor Orange 560 produced detectable results with threshold cycles (Cts) ranging from 24 to 25 (Fig 1). Reactions with Quasar 570, and CAL Fluor Red (590 and 610) were not detectable in real-time, however PCR products were detected by electrophoresis.

Discussion and Conclusions

The results demonstrate that the Spartan DX is able to perform real-time PCR using TaqMan probes with FAM, CAL Fluor Gold 540, and CAL Fluor Orange 560 fluorophores. Due to their overlapping excitation and emission wavelengths, we predict that the Spartan DX should also be compatible with other green and yellow emitters, such as VIC, JOE, HEX and TET fluorophores.