Efficient Real-Time PCR on the Spartan DX-12™ Instrument

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Here we demonstrate that the Spartan DX-12™ instrument is capable of efficient real-time PCR.

Introduction

In a perfectly efficient PCR reaction, the number of amplicons should double at each cycle (1). Practically this is not always the case as there are many variables within a reaction that can affect its efficiency, including the presence of inhibitors, enzyme kinetics, and primer/probe interactions (2). However, PCR efficiency is generally considered to be an effective indicator of instrument performance.

The purpose of this study was to determine the real-time PCR efficiency across several Spartan DX-12™ instruments.

Materials and Methods

DNA preparation

A synthetic plasmid construct was designed in-house and produced for Spartan Bioscience by GENEART Inc. (Toronto). Plasmid DNA received at a stock concentration of 1.5 mg/ml was reconstituted to 1.5 mM stock in sterile water, and stored in aliquotes at -20°C.

Input DNA

Plasmid DNA was diluted to a concentration of 0.6 ng/µl. Serial dilutions were prepared across several logs (10^1 – 10^{10}) in sterile water. Since 2 µl of DNA was used per reaction, this corresponds to input DNA amounts of 12 ng, 1.2 ng, 120 pg, 12 pg, 1.2 pg, 120 fp, 12 fg, 1.2 fg, 120 ag, 12 ag, 1.2 ag, 0.12 ag. The corresponding copy numbers were: $4.04x10^8$, $4.04x10^7$, $4.04x10^6$, $4.04x10^5$, $4.04x10^4$, $4.04x10^3$, $4.04x10^2$, 40.4, 4.04, $4.04x10^{-1}$, $4.04x10^{-2}$.

Real-time PCR

Oligonuceotide primers and probe designed against the Norovirus Open Reading Frame exon 1-2 junction (ORF 1-2) were used (3, 4). The primer & probe sequences are shown in Table 1.

Components of the real-time amplification mixture are listed in Table 1. Samples were amplified in Spartan Tubes (20µl PCR Tube Assembly, Tubes - Cat. No.01004153 and Caps - Cat. No. 01004155), and amplification was performed on two Spartan DX-12™ instruments. Table 3 shows the cycling parameters used (2-temperature program).

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
COG2 primers	CAR gAR BCN ATg TTY AgR Tgg ATg Ag	TCg ACg CCA TCT TCA TTC ACA	98
COG2 probe	6-CAL Fluor® Red 610 -Tgg gAg ggC gAT CgC AAT CT-BHQ2		

6-FAM = 6-carboxy-fluorescein, BHQ = Black Hole Quencher

Table 1. Primer/probe sequences and amplicon sizes.

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 (Molecular Probes)	0.5 X
PCR primers (IDT)	0.2 µM
Template DNA	2 µl
Sterile water	up to 20 µl
Total reaction volume	**20 µl**

Table 2. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	30 s	1
Denaturation	95°C	25 s	40
Annealing/extension	55°C	25 s	40

Table 3. Cycling parameters.

DNA analysis

Fluorescence data was transferred from the Spartan DX-12 and graphed using the Spartan Graphing & Analysis Software (ver. 3.10).

Results

Figure 1 (A, C) shows plots of normalized fluorescence versus cycle number for two separate Spartan DX-12™ instruments. Successful amplification curves are shown across 7-8 logs. This corresponds to 12 ng-120 ag of DNA, estimated to be 4.04x10^9-40.4 copies of template. Figure 1 (B, D) show the plots of crossing point versus the logarithm of copy number, as plotted by the Spartan Graphing and Analysis Software. Efficiency, R and R^2 values are 1.949, 0.999, 0.999 for Instrument 1 and 1.947, 0.999, 0.999 for Instrument 2.

Discussion and Conclusions

The results demonstrate that the Spartan DX-12™ has a dynamic range of at least 8 logs, and is capable of achieving PCR efficiencies approaching the theoretical maximum of 100 percent.

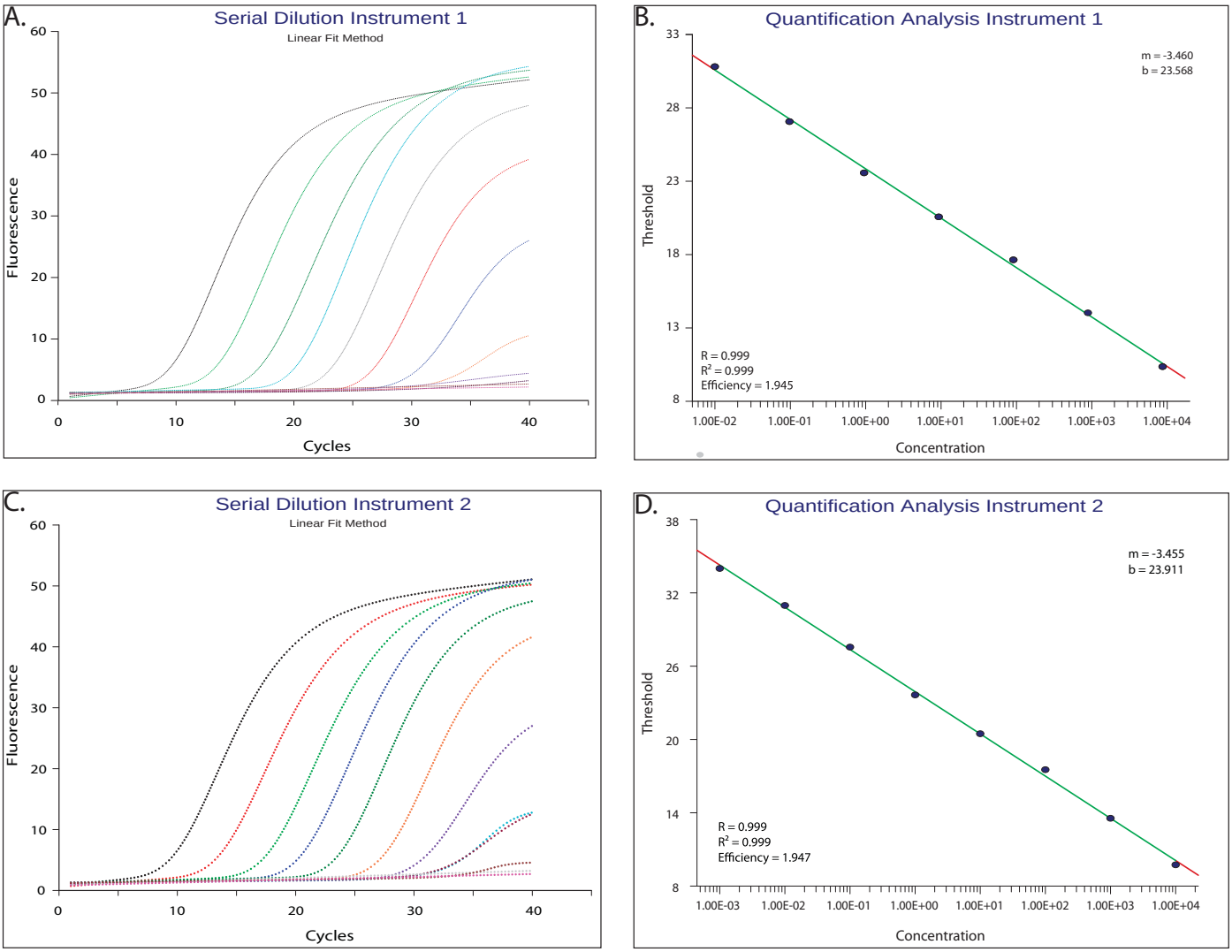

Figure 1. Real-Time PCR results for serial dilutions, on two seperate instruments showing 7-8 logs dynamic range.

Although the Spartan DX-12™ is described as a "semiquantitative" device due to the limited number of wells, these results demonstrate that the Spartan DX-12™ has excellent quantitative performance. The instrument can easily generate and export Real-Time PCR standard curve analyses and is capable of detecting DNA down to the theoretical limits of 1 - 3 copies at close to the theoretical maximum efficiency of 100%.

References

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