Efficient real-time PCR

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The Spartan DX™ is capable of achieving real-time PCR efficiencies approaching the theoretical maximum of 100 percent.

Introduction

According to PCR theory, the number of amplicons should double with each cycle if the reaction is perfectly efficient (Ref 1). In practice, PCR efficiency may be less than 100 percent because of factors such as inhibitors and differences in primers, probes and enzyme kinetics (Ref 2).

The Spartan DX performs real-time PCR using a heating method where reaction tubes are placed in partial contact with fixed-temperature heat sources. Compared to a conventional Peltier-controlled heat block thermal cycler, significant time is saved by not having to increase or decrease the temperature of the block.

The purpose of this study was to determine real-time PCR efficiency of the Spartan DX.

Materials and Methods

DNA preparation

Plasmid DNA was purified by GenElute™ Maxiprep kit (Sigma). The DNA purity and concentration was assessed spectrophotometrically and verified by ethidium bromide staining.

Input DNA

The plasmid DNA (7,300 bp) was determined to have a concentration of 62.5 µg/µl. Serial dilutions were prepared with dilution factors of 2^3, 2^6, 2^9, 2^{12}, 2^{15}, 2^{18}, 2^{21}, 2^{24}, 2^{27} and 2^{30}. Since 1 µl of DNA was used per reaction, these dilutions corresponded to input DNA amounts of 7.81 ng, 977 pg, 122 pg, 15.3 pg, 1.91 pg, 238 fg, 29.8 fg, 3.73 fg, 466 ag and 58 ag, respectively. The corresponding copy numbers were: 9.8×10^8, 1.2×10^8, 1.5×10^7, 1.9×10^6, 2.4×10^5, 3.0×10^4, 3.7×10^3, 4.7×10^2, 58 and 7, as calculated with the following formula:

$$\frac{X \text{ DNA (g/µl)} \times 6.022 \times 10^{23}}{\text{plasmid length (bp)} \times 660 \text{ (g/mol)}} = Y \text{ molecules/µl}$$

Real-time PCR

Oligonucleotide primers were designed against the rat *HTR1A* gene. The forward primer sequence was 5′-gcg gtg ccg acg aag t-3′ and the reverse primer was 5′-gcc atc gcg cta gac agg ta-3′. The expected amplicon size was 360 bp.

Components of the real-time PCR amplification mixture are listed in Table 1. SYBR Green I dye (Invitrogen, Cat. No. S-7563) was used for fluorescent detection. Samples were amplified in 0.2 ml flat-cap PCR tubes (Axygen, Cat. No. PCR 02C) and topped with 15 µl of mineral oil (Biotools, Cart No. 20.032) to prevent evaporation. Real-time PCR was performed using the Spartan DX instrument. Table 2 shows the cycling parameters for a two-temperature program which combines the primer annealing and extension steps. Amplification was confirmed by agarose gel electrophoresis using 5 µl of the PCR products.

Component	Final amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1 X
$MgCl_2$ (Invitrogen)	3 mM
dNTP mix (Invitrogen)	0.2 mM
Taq DNA polymerase (Invitrogen)	1 U
SYBR Green I (Molecular Probes)	0.5 X
PCR primers (IDT)	0.5 µM
Template DNA	1 µl
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	97°C	60 s	1
Denaturation	97°C	45 s	50
Annealing/extension	58°C	45 s	50

Table 2. Cycling parameters.

DNA analysis

Fluorescence data was downloaded from the Spartan DX and graphed using Microsoft Excel®. Fluorescence amplitudes were normalized to 100 percent ([observed fluorescence - initial fluorescence] ÷ maximum fluorescence

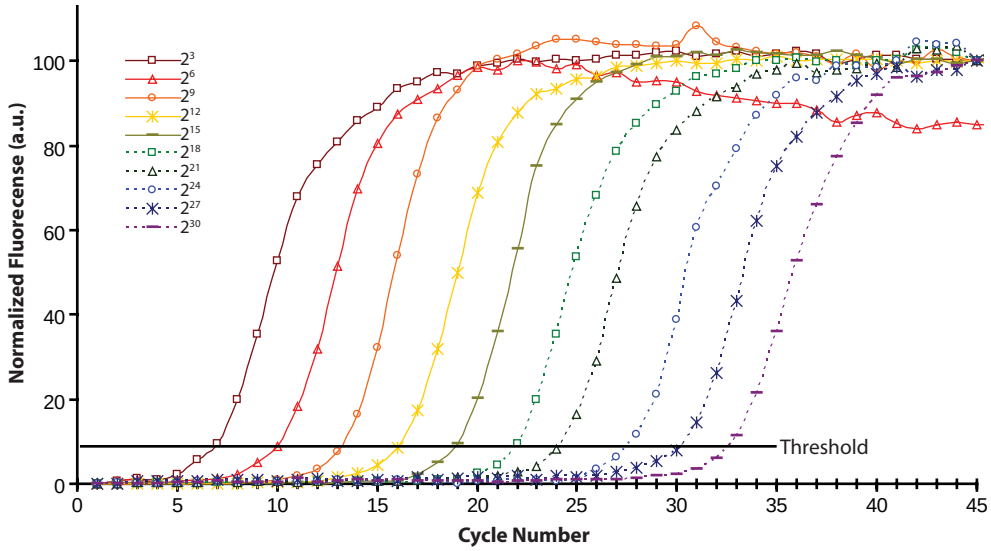

Figure 1. Real-time PCR results for 10 dilutions of plasmid DNA (2^3, 2^6, 2^9, 2^{12}, 2^{15}, 2^{18}, 2^{21}, 2^{24}, 2^{27} and 2^{30}). Normalized fluorescence in arbitrary units (a.u.) is plotted against cycle number.

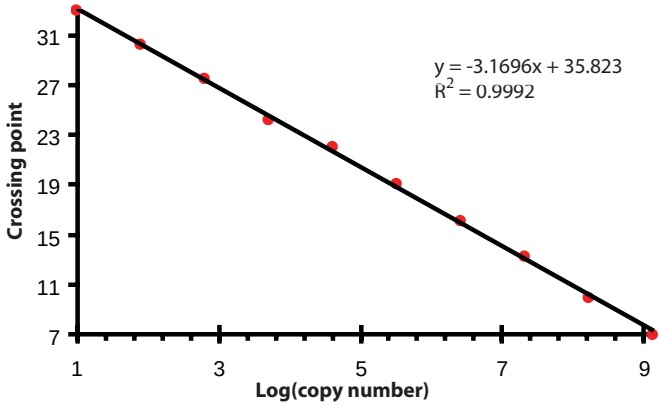

Figure 2. Plot of crossing point values versus logarithm of plasmid copy number. The linear equation of best fit is shown as well as the regression coefficient (R^2).

× 100%). The crossing point values for each concentration were determined by examining the coordinates of the curve where it crosses the threshold line (Ref 3). The crossing point values for each concentration were plotted against the logarithm of copy number to assess PCR efficiency.

Results

Figure 1 shows a plot of normalized fluorescence versus cycle number. Successful amplification curves can be seen with dilutions ranging from 2^3 to 2^{30}. This corresponds to DNA concentrations from 7.81 ng to 58 ag, or copy numbers from 9.8×10^8 to 7 copies. Gel electrophoresis showed the expected 360 bp fragment for all dilutions.

Figure 2 shows a plot of crossing point versus the logarithm of copy number. Linear regression analysis determined that the slope of the line was -3.1696 which resulted in a calculated reaction efficiency of 106.8% and an amplification coefficient of 2.07 (http://www.stratagene.com/techtoolbox/calc/qpcr_slope_eff.aspx).

Discussion and Conclusions

The results demonstrate that the Spartan DX has a detection range of 7.81 ng to 58 ag. We conclude that the Spartan DX is capable of achieving PCR efficiencies approaching the theoretical maximum of 100 percent.

References

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