Thermal cycler sample amplification uniformity: a comparison of several models

Introduction

Well-to-well amplification uniformity in a thermal cycler is a critical factor that can affect PCR results and their analysis. Without uniform reaction conditions across the block, users cannot make robust conclusions when comparing data obtained from wells at two different locations. This study measures the reaction yield of several thermal cyclers using post-reaction absolute fluorescence. Well-to-well amplification uniformity is then assessed by calculating the coefficient of variation (CV) of fluorescence readings obtained by each thermal cycler.

Materials and methods

The instruments tested in this study are shown in Table 1. The same equipment, methods, and reagents were used for each instrument.

Table 1. Instruments tested for amplification uniformity.

Manufacturer	Model name	Cat. No.
Thermo Fisher Scientific	Applied Biosystems™ 2720 Thermal Cycler	4359659
Thermo Fisher Scientific	Applied Biosystems™ MiniAmp™ Thermal Cycler	A37834
Thermo Fisher Scientific	Applied Biosystems™ MiniAmp™ Plus Thermal Cycler	A37835
Thermo Fisher Scientific	Applied Biosystems™ SimpliAmp™ Thermal Cycler	A24811
Thermo Fisher Scientific	Applied Biosystems™ ProFlex™ 96-Well PCR System	4484075
Agilent	SureCycler™ 8800 Thermal Cycler	G8800A
Bio-Rad	C1000 Touch™ Thermal Cycler with 96-Well Fast Reaction Module	185-1196
Bio-Rad	T100™ Thermal Cycler	186-1096
Eppendorf	Mastercycler™ Nexus Gradient	6331 000.017
Eppendorf	Mastercycler™ Pro S	6325 000.510
SensoQuest	Labcycler Gradient	011-101, 012-103
Takara	Dice™ Touch	TP350

Sample preparation

A single bulk reaction was prepared using Applied Biosystems™ AmpliTaq Gold™ 360 Master Mix (Cat. No. 4398886) according to the standard protocol. Lambda DNA standard (component C from the Invitrogen™ Quant-iT™ PicoGreen™ dsDNA Assay Kit, Cat. No. P7589) was used as the template at a final concentration of 0.01 ng/µL, with forward primer (5′-GATGAGTTCGTGTCCGTACAACT-3′) and reverse primer (5′-ACGGCTGCACGGAGTTCAGTATG-3′) at 0.2 µM. The bulk reaction was tested at 10 µL or 100 µL testing volumes using nonoptical 96-well plates. The following thermal profile was used for all instruments in this study: a primary stage at 94°C for 10 minutes, a secondary stage of 25 cycles at 94°C for 15 seconds and 70°C for 90 seconds, and a final stage at 72°C for 7 minutes.

Data acquisition and analysis

After completion of thermal cycling, Applied Biosystems™ *Power* SYBR™ Green PCR Master Mix (Cat. No. 4368577) was added to each reaction and incubated for 10 minutes. The reactions were then transferred to an Applied Biosystems™ MicroAmp™ Optical 96-Well Reaction Plate (Cat. No. N8010560) for fluorometric analysis on the Applied Biosystems™ ViiA™ 7 Real-Time PCR System (Cat. No. 4453534). Amplification uniformity was calculated using the following formula:

$$\text{CV (\%)} = \frac{\text{Standard deviation of SYBR Green signal over 3 runs}}{\text{Average SYBR Green signal over 3 runs}} \times 100\%$$

Results

The average fluorescence intensity was obtained from each thermal cycler over two reaction volumes in 96-well plates. Figure 1 shows the CV for each reaction volume. The CV group average of 9.64% is shown as a red line.

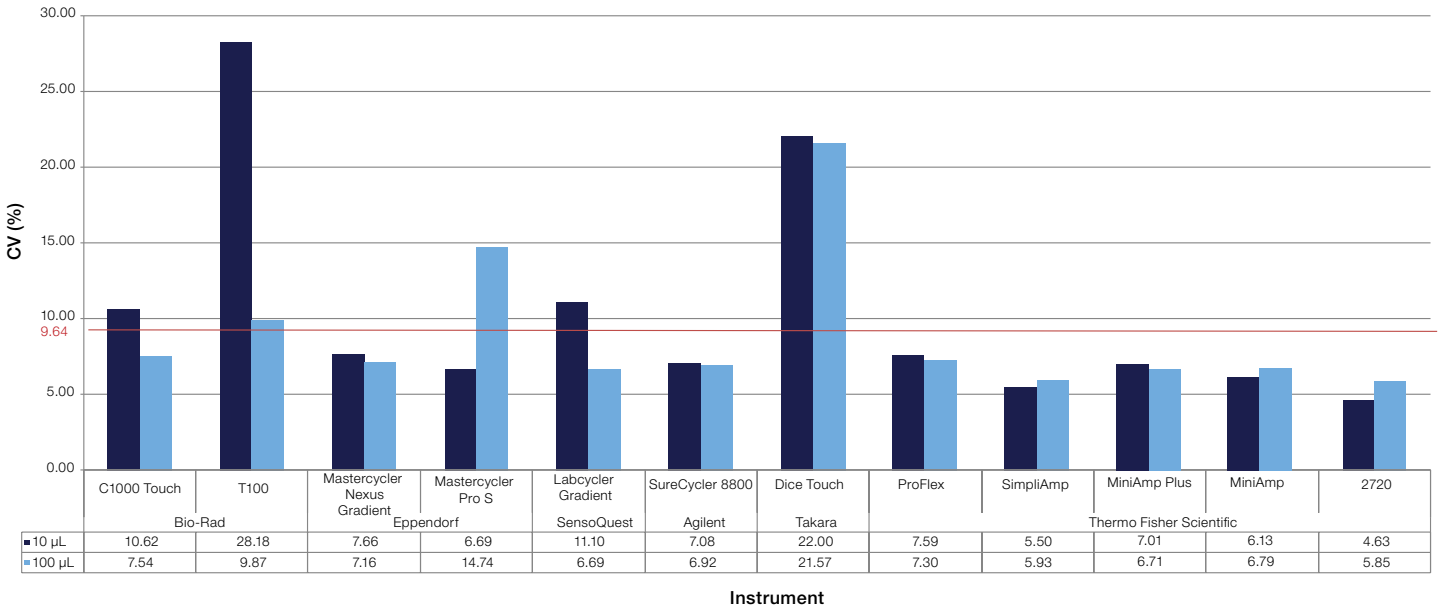

Figure 1. Uniformity of PCR yields as measured by CV.

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Discussion

The ability of a thermal cycler to uniformly amplify replicate reactions in multiple wells of a plate is a pivotal requirement that users may overlook when designing experiments. In this study, we have shown that PCR amplification uniformity varies between thermal cyclers. This was demonstrated by side-by-side comparisons of reactions of the same chemical formulation.

For the best results, we recommend using a thermal cycler with good well-to-well uniformity (as indicated by low CV). The results for each volume suggest that the Applied Biosystems™ thermal cyclers meet this criterion.

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