Assessment of PCR plate performance in the Automated Thermal Cycler

Introduction

Consistent amplification uniformity during PCR is vital to making robust conclusions when comparing data obtained from wells at two different locations. This study demonstrates the effect that PCR plate consumables can have on amplification uniformity in the Applied Biosystems™ Automated Thermal Cycler (ATC) by comparing coefficient of variation (CV) values and post-reaction fluorescence surface plots across two types of full-skirted 96-well plates.

Materials and methods

The top-selling full-skirted 96-well PCR plates, shown in Table 1, were selected for evaluation on the ATC. The same equipment, methods, and reagents were used to set up each PCR reaction. All PCR plate consumables were tested using the ATC.

A single bulk reaction was prepared using Applied Biosystems™ *Power* SYBR™ Green PCR Master Mix (Cat. No. 4367660) 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 a template at a final concentration of 0.01 ng/µL, with lambda forward primer (5´-GATGAGTTCGTGTCCGTACAACT-3´) and lambda reverse primer (5´-ACGGCTGCACGGAGTTCAGTATG-3´) each at 0.2 µM concentration. The bulk reaction was tested at 10 µL and 100 µL volumes to compare different full-skirted 96-well plates. The thermal profile in Table 2 was used for each reaction.

Table 1. Full-skirted 96-well PCR plates tested for amplification uniformity.

Manufacturer	Description	Cat. No.
Thermo Fisher Scientific	Applied Biosystems™ MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, yellow	A31730
Eppendorf	Eppendorf™ twin.tec™ PCR Plate 96, skirted, 150 µL, PCR clean, yellow	951020427 (US), 0030128656 (EU)

Table 2. Thermal profile for PCR on the ATC.

Stage	Temperature (°C)	Time (min:sec)	Number of cycles
1	94	10:00	1
2	94	00:15	25
	70	01:30	
3	72	07:00	1

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Data acquisition and analysis

After completion of thermal cycling, the PCR reactions were transferred to a semi-skirted 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 as the CV using the following formula:

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

Results

The average fluorescence intensity was measured for each full-skirted 96-well PCR plate across three runs and two reaction volumes, 10 µL and 100 µL. The amplification uniformity was calculated as CV, and results are shown in Table 3 and Figure 1.

Table 3. Average fluorescence intensity and CV for full-skirted 96-well PCR plates tested in triplicate with low and high reaction volumes.

Sample volume	PCR plate type	Average fluorescence intensity	CV (%)
10 µL (low)	MicroAmp EnduraPlate	4,400,778	4.94
	twin.tec	4,347,380	5.36
100 µL (high)	MicroAmp EnduraPlate	4,677,819	5.59
	twin.tec	4,677,741	5.41

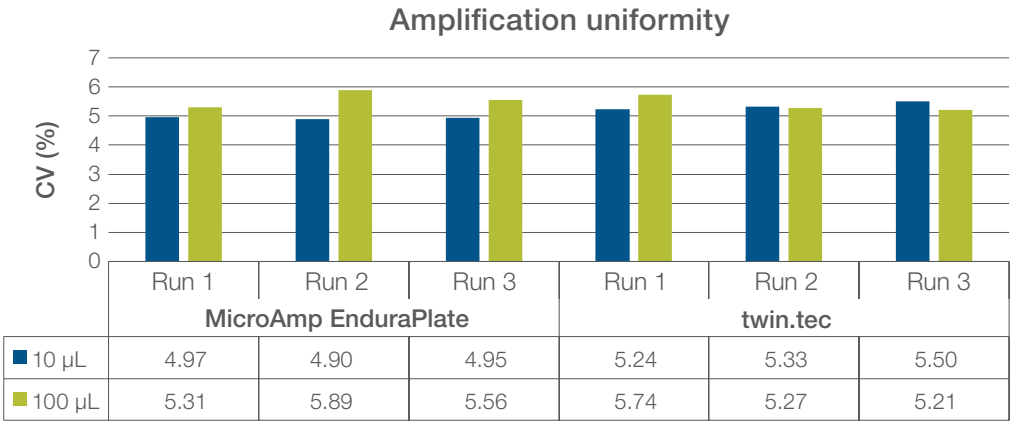

Figure 1. Amplification uniformity in full-skirted 96-well PCR plates on the ATC, as measured by CV.

A surface plot for each PCR plate was generated using the fluorescence intensity results, to gain additional information on well-to-well uniformity across the plate. Although it is common to see variation in fluorescence signal across the surface of a plate, it is important to identify if there are any significant outliers that may impact the ability to make robust conclusions when comparing data from two different well locations.

Figure 2 shows fluorescence intensity surface plots for the 10 µL reactions on the MicroAmp EnduraPlate full-skirted plate from the front and back orientation of the plate. For comparison, Figure 3 shows the same measurements for the twin.tec PCR plate. Results show a significant reduction in fluorescence signal for well A2 (circled in red) of the twin.tec PCR plate with the low reaction volume, while the MicroAmp EnduraPlate full-skirted plate shown in Figure 2 displays more uniformity in fluorescence signal across the plate. Fluorescence intensity values from selected wells, and their corresponding plate averages from a single PCR run, are shown in Table 4.

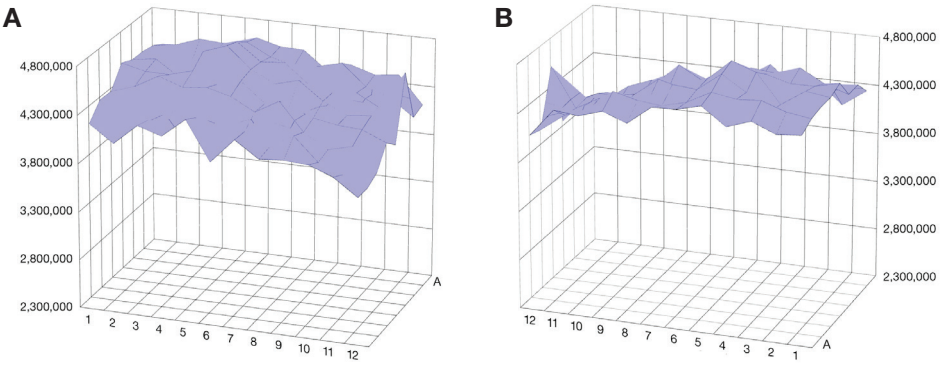

Figure 2. Fluorescence intensity surface plots for the MicroAmp EnduraPlate full-skirted plate with 10 µL reactions. Data are shown for the **(A)** front and **(B)** back orientation of the plate.

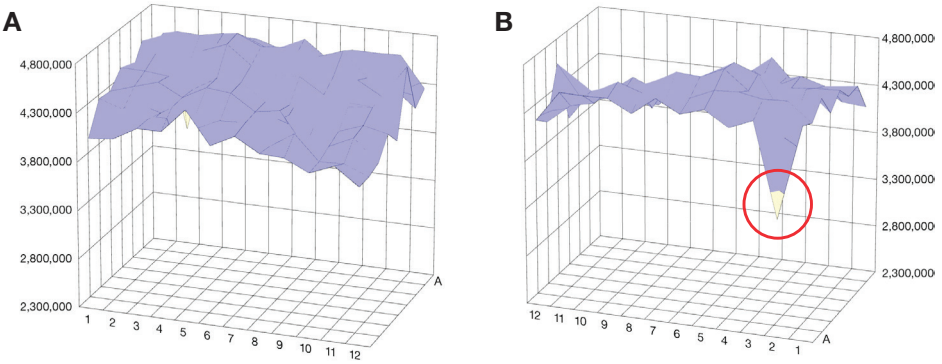

Figure 3. Fluorescence intensity surface plots for the twin.tec PCR plate with 10 µL reactions. Data are shown for the **(A)** front and **(B)** back orientation of the plate.

Table 4. Comparison of fluorescence intensity and CV across individual well locations.

		Fluorescence intensity					CV (%)	
Sample volume	PCR plate type	Plate average	Well A1	Well A2	Well A3	Well A4	Plate average	CV of well A2 to plate average
10 µL	twin.tec	4,360,442	4,513,613	3,528,115	4,508,145	4,406,169	5.24%	13.50%
	MicroAmp EnduraPlate	4,349,595	4,428,231	4,406,264	4,508,960	4,423,717	4.90%	0.90%
100 µL	twin.tec	4,715,719	5,036,166	5,035,574	5,184,925	5,083,492	5.74%	4.77%
	MicroAmp EnduraPlate	4,775,425	5,240,207	5,128,171	5,071,795	5,018,497	5.31%	5.22%

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A comparison of the two plates highlights that the MicroAmp EnduraPlate full-skirted plate does not have the robotic slot that is present in the plate skirt in front of well A2 in the twin.tec PCR plate, as shown in Figure 4. This suggests that robotic slots in a plate's skirt may introduce environmental effects to the adjacent wells and impact performance during PCR.

The signal reduction observed in well A2 in the twin.tec plate with the 10 µL reaction volume was not observed with the 100 µL reaction volume. Figure 5 shows the fluorescence intensity surface plots for both MicroAmp EnduraPlate and twin.tec PCR plates at the high reaction volume.

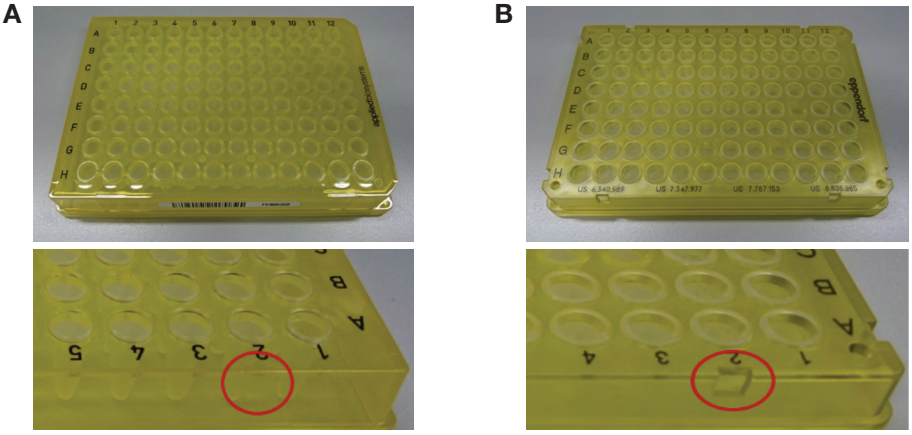

Figure 4. Comparison of PCR plates. (A) MicroAmp EnduraPlate full-skirted plate, with a view of the skirt in front of well A2. **(B)** twin.tec PCR plate, with a view of the robotic slot in the skirt in front of well A2.

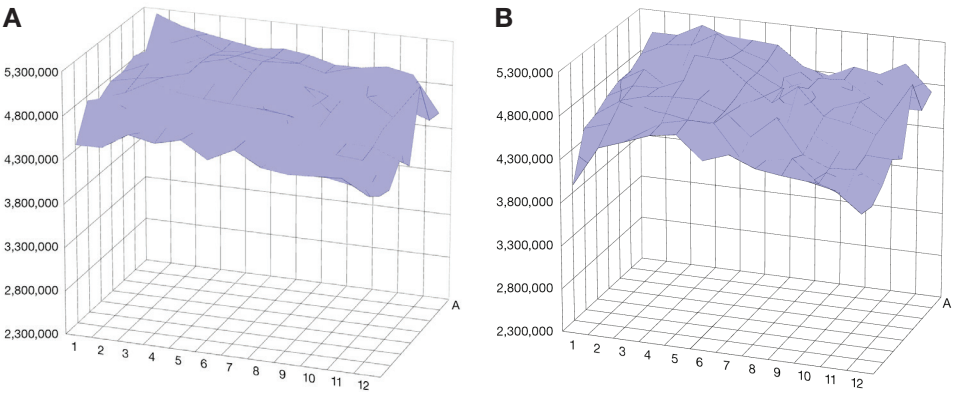

Figure 5. Fluorescence intensity surface plots for 100 µL reactions. Data are shown for **(A)** MicroAmp EnduraPlate and **(B)** twin.tec PCR plates.

Discussion

Consistent amplification uniformity during PCR is vital to making robust conclusions when comparing data obtained from wells at two different locations. In this study, we have shown the differences in post-reaction fluorescence surface plots across two full-skirted 96-well plates with 10 µL reactions. These results highlight the importance of PCR plate selection on the ATC to help ensure amplification uniformity across wells.

The ATC features a unique design element with its automated lid. The heated cover slides forward and retracts over the thermal block to provide hands-free operation that is compatible with a liquid handler or plate stacker. This design feature may cause full-skirted 96-well plates containing robotic slots, such as the twin.tec PCR plate, to experience environmental differences in specific well positions that may impact PCR performance.

For best results, we recommend using PCR plate consumables that are designed and confirmed for use with the ATC instrument, such as the MicroAmp EnduraPlate Optical 96-Well Full-Skirted Plates with Barcode. These plates provide amplification uniformity (as indicated by low CV) and display consistency in the fluorescence intensity surface plots for both 10 µL and 100 µL reaction volumes.

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