A Model Comparability Study of the C1000 Touch and PTC Tempo Thermal Cyclers

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Abstract

A comparability, or bridging, study is often required when changes are made to laboratory protocols and/or equipment in an existing process. The study presented here outlines experiments comparing the thermal performance between the PTC Tempo and C1000 Touch Thermal Cyclers across all four formats: 96-well, 96–deep well, 384-well, and 48/48-well. The performance of the thermal cyclers was compared across multiple applications ranging from cDNA synthesis to conventional and gradient PCR.

Introduction

Ubiquitous in laboratory settings, thermal cyclers are used to perform PCR functions across a wide range of applications, including sequencing, cloning, genotyping, mutation detection, protein melt assays, and many others. The increasing complexity of PCR applications requires that thermal cyclers be continually improved to achieve ever higher levels of performance. To meet the demands of complex PCR applications, the PTC Tempo Thermal Cycler was designed to include precise temperature control and enable flexible thermal gradient configuration. Multiple block formats offer a range of throughput and volume capabilities.

As shown in Table 1, the PTC Tempo Thermal Cycler builds on the performance of its predecessor, the C1000 Touch Thermal Cycler, adding user-friendly features to the 384-well, 96-well, and 96–deep well, such as an automated lid (capable of autosensing plates and tubes), an LED light display, audible status notifications, and additional connection options, including cloud (BR.io cloud platform), WiFi, USB, and Ethernet connections. While the PTC Tempo 48/48 Thermal Cycler has a non-automated lid, it includes all prior new features. Overall, the PTC Tempo Thermal Cycler maintains the same user access controls for user management but with enhanced security and reporting features.

Experimental Design

This comparability study demonstrates that the PTC Tempo and C1000 Touch Thermal Cyclers are functionally equivalent based on functional testing results. The parameters for thermal uniformity, accuracy, and ramp rate are all comparable. The PTC Tempo 96 Thermal Cycler was compared to the C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module. The PTC Tempo Deepwell Thermal Cycler was compared to the C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module. The PTC Tempo 384 Thermal Cycler was compared to the C1000 Touch Thermal Cycler with 384-Well Reaction Module. Lastly, the PTC Tempo 48/48 Thermal Cycler was compared to the C1000 Touch Thermal Cycler with Dual 48/48 Fast Reaction Module. For these comparisons, cDNA synthesis efficiency was measured using multiple Bio-Rad kits. Uniformity was assessed using SYBR® Green– and gel-based assays. Gel electrophoresis was used to visualize the gradient effects on PCR product integrity and ability to produce PCR products of various sizes in high- and low-volume reactions.

Materials and Methods

cDNA Synthesis Comparison

A reverse transcription quantitative PCR (RT-qPCR) assay was developed using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., catalog #1708890) and Reliance Select cDNA Synthesis Kit (Bio-Rad, #12012802). The assay was used to compare the amplification efficiency of cDNA synthesis between the PTC Tempo 96 Thermal Cycler (Bio-Rad, #12015382) and C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module (Bio-Rad, #1851196), between the PTC Tempo Deepwell Thermal Cycler (96-well; Bio-Rad, #12015392) and C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module (Bio-Rad, #1851197), between the PTC Tempo 384 Thermal Cycler (Bio-Rad, #12015394) and C1000 Touch Thermal Cycler with 384-Well Module (Bio-Rad, #1851138), and between the PTC Tempo 48/48 Thermal Cycler (Bio-Rad, #12015309) and C1000 Touch Thermal Cycler with Dual 48/48 Fast Reaction Module (Bio-Rad, #1851148). Specifically, the

RT-qPCR assay was used to determine whether the old and new thermal cycler models generate equivalent levels of cDNA in the initial reverse transcription reaction. The sensitivity of the RT-qPCR assay enables the detection of minute differences in the quantity or quality of cDNA produced using the resulting quantification cycle (Cq). Relative differences in cDNA abundance should be minimal under equivalent reaction conditions. Differences were minimal for four housekeeping genes (*GAPDH*, *LDHA*, *PPIA*, and *UBC*) tested using a Bio-Rad Custom PrimePCR Real-Time PCR Plate containing standardized RT-qPCR assays.

Conventional PCR Uniformity

Thermal cycler uniformity was evaluated by running amplifications on three instruments of each model. After cycling in a PTC Tempo or C1000 Touch Thermal Cycler, results were visualized after electrophoresis with agarose gels stained with Ethidium Bromide Solution (EtBr; Bio-Rad, #1610433) and by running a SYBR® Green assay; endpoint fluorescence intensities of the amplification of each well were evaluated.

For the SYBR® Green assay, samples containing reaction mixtures with SYBR® Green Dye were first read in a real-time PCR system to obtain the background values for later subtraction. The samples were then amplified on the PTC Tempo Thermal Cyclers using a standard PCR protocol, followed by plate reads in an appropriate real-time PCR system to obtain the post-amplification data as follows. For the 96-well and 48/48-well module experiments, plates were read in a CFX Duet Real-Time PCR System (Bio-Rad, #12016265). For the 384-well module experiments, plates were read in a CFX Opus 384 Real-Time PCR System (Bio-Rad, #12011452). Lastly, for the 96–deep well module experiments, the plates were read in a CFX Opus Deepwell Real-Time PCR System (Bio-Rad, #12016658). Coefficients of variation (CV) for replicates in the SYBR® Green assay were then assessed in the indicated volumes for each thermal cycler model using different consumables.

When assessing the amplified products, the four C1000 Touch and four PTC Tempo Thermal Cycler configurations all completed validation with Microseal™ 'B' PCR Plate Sealing Film, adhesive, optical (Bio-Rad, #MSB1001) as a plate seal. For each configuration type, three C1000 Touch and three PTC Tempo units were used to test the Microseal 'B' PCR Plate Sealing Film.

Various product combinations were used for evaluating consumables. For dual 48/48-well thermal cyclers, one run was performed on the 10 µl reaction using Multiplate 48-Well PCR Plates, high profile, unskirted, clear (Bio-Rad, #MLP4801), and three runs were performed on the 20 µl reaction using #MLP4801 with #MSB1001. For 384-well thermal cycler systems, three runs were performed on the 10 µl reaction with three C1000 Touch and three PTC Tempo units, using Hard-Shell™ 384-Well PCR Plates, thin wall, skirted, clear/clear (Bio-Rad, #HSP3801) with #MSB1001.

For 96-well fast systems, Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted, white/clear (Bio-Rad, #HSP9601) and Multiplate 96-Well PCR Plates, low profile, unskirted, clear (Bio-Rad, #MLL9601) were used. For 96–deep well systems, #HSP9601 and Multiplate 96-Well PCR Plates, high profile, unskirted, clear (Bio-Rad, #MLP9601) were used.

For PTC Tempo 48/48 Thermal Cycler experiments, Multiplate 48-Well PCR Plates, low profile, unskirted, clear (Bio-Rad, #MLL4801) were used with #MSB1001 or 0.2 ml Domed PCR 8-Cap Strips, clear (Bio-Rad, #TCS0801). Additionally, MLP4801 with #MSB1001 was also used in this system configuration. #HSP3801 was used with #MSB1001 for PTC Tempo 384 Thermal Cycler experiments. For PTC Tempo 96 Thermal Cycler experiments, #HSP9601 and #MLL9601 were used, whereas for PTC Tempo Deepwell Thermal Cycler experiments, #HSP9601 and #MLP9601 were used. The Wide Mini-Sub Cell GT Horizontal Electrophoresis System, 15 x 10 cm tray (Bio-Rad, #1704468) was used for nucleic acid separation. Gels were imaged on a ChemiDoc™ MP Imaging System (Bio-Rad, #12003154).

For determining the percent CV for the DNA gel electrophoresis technique, the contents of the 384-well uniformity plate were transferred to the first and second rows of eight 1% TAE ReadyAgarose 96 Plus Gels, 15.6 x 10 cm, 4 x 26-well, with ethidium bromide (Bio-Rad, #1613063). The average uniformity was determined by calculating the CV for each gel, followed by averaging the individual CV values.

Conventional PCR Product Size

Consistent thermal cycling during a PCR reaction is essential for generating appropriately sized fragments from sequential rounds of amplification. To evaluate performance, small (102 bp), medium (1.3 kb), and large (10 kb) PCR products were amplified (Appendix). Triplicate tests were performed at several different well locations on the block using 10 and 50 µl volumes for 96-well thermal cyclers, 10 and 125 µl for deep well thermal cyclers, 3 and 20 µl for 384-well thermal cyclers, and 10 and 50 µl for 48/48 thermal cyclers.

Gradient-Based PCR

A thermal gradient ranging from 46 to 70ºC was used to evaluate a PCR reaction run in triplicate; the 24-degree span included temperatures above, below, and at the optimal annealing temperature of the reaction. PCR reactions at temperature settings within the optimal annealing temperature range were expected to produce a single 100 bp product. Reactions below the optimal annealing temperature range were expected to lead to nonspecific, or promiscuous, primer binding, producing multiple fragment sizes. Finally, those above the optimal range were expected to lead to progressively smaller amounts of product due to the inhibition of primer binding.

Results

cDNA Synthesis Comparison

The results of the RT-qPCR assays using iScript and Reliance Select cDNA Synthesis Kits for one C1000 Touch Thermal Cycler and three different PTC Tempo Thermal Cyclers of each block format are shown in Figure 1. Minimal fold changes in cDNA abundance were detected across thermal cyclers and block formats, indicating consistent production of cDNA prior to the reverse transcriptase reaction.

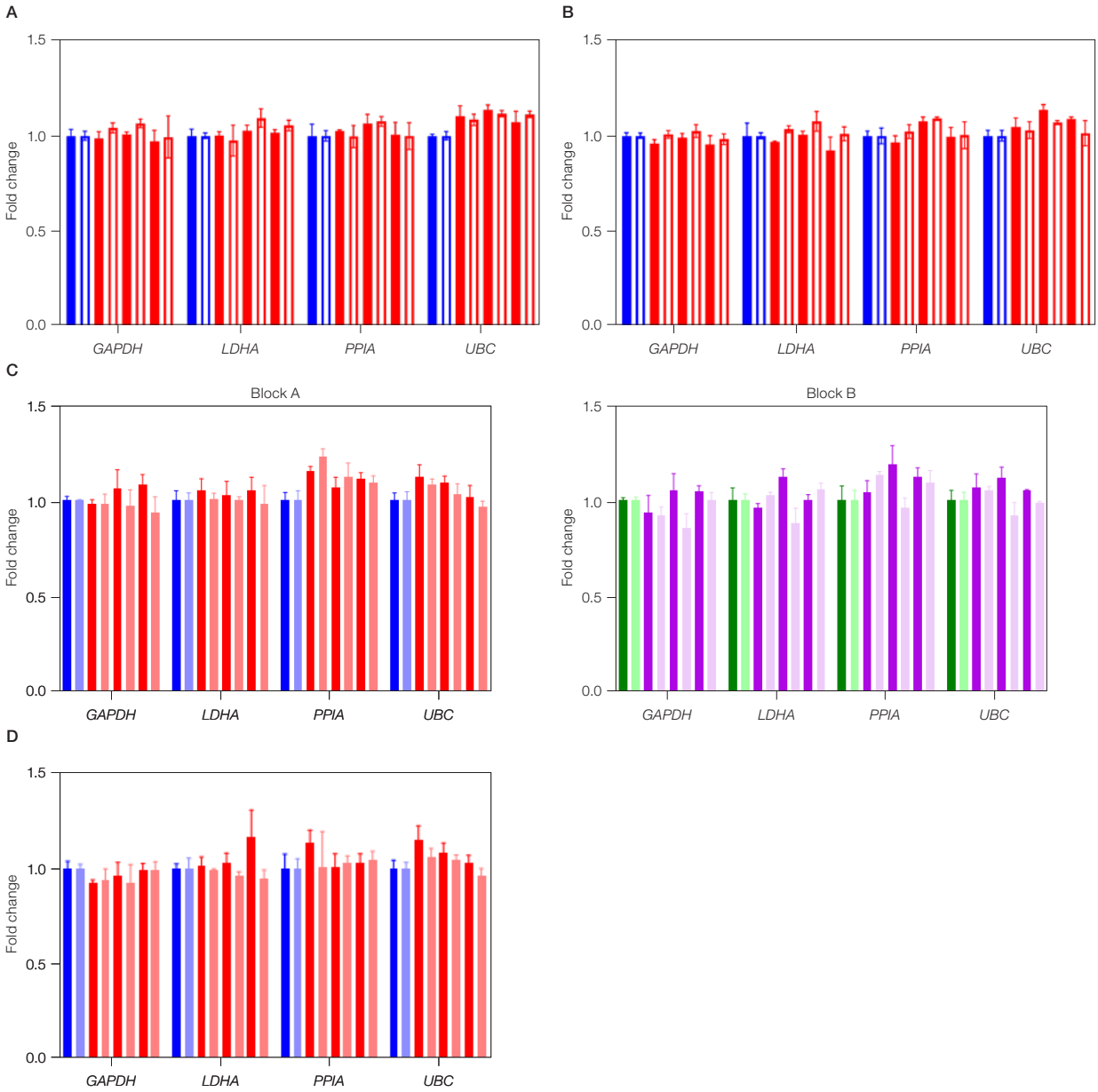


Fig. 1. Amplification efficiency using iScript and Reliance Select cDNA Synthesis Kits for cDNA generation. Minimal differences were observed across thermal cyclers and block formats. **A**, Cq fold changes for one C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module and three PTC Tempo 96 Thermal Cyclers; **B**, Cq fold changes for one C1000 Touch Thermal Cycler with 96–Deep Well Module and three PTC Tempo Deepwell Thermal Cyclers; **C**, Cq fold changes for one C1000 Touch Thermal Cycler with Dual 48/48 Fast Reaction Module and three PTC Tempo 48/48 Thermal Cyclers, shown as Blocks A and B; **D**, Cq fold changes for one C1000 Touch Thermal Cycler with 384-Well Reaction Module and three PTC Tempo 384 Thermal Cyclers.

A and **B**, C1000 Touch Thermal Cycler with iScript Kit (■); C1000 Touch Thermal Cycler with Reliance Kit (□); PTC Tempo Thermal Cycler with iScript Kit (■); PTC Tempo Thermal Cycler with Reliance Kit (□). **C**, Block A: C1000 Touch 48/48 Thermal Cycler with iScript Kit (■); C1000 Touch 48/48 Thermal Cycler with Reliance Kit (■); PTC Tempo 48/48 Thermal Cycler with iScript Kit (■); PTC Tempo 48/48 with Reliance Kit (■). Block B: C1000 Touch 48/48 Thermal Cycler with iScript Kit (■); C1000 Touch 48/48 Thermal Cycler with Reliance Kit (■); PTC Tempo 48/48 Thermal Cycler with iScript Kit (■); PTC Tempo 48/48 Thermal Cycler with Reliance Kit (■). **D,** C1000 Touch 384-Well Thermal Cycler with iScript Kit (■); C1000 Touch 384-Well Thermal Cycler with Reliance Kit (■); PTC Tempo 384 Thermal Cycler with iScript Kit (■); PTC Tempo 384 Thermal Cycler with Reliance Kit (■).

Error bars indicate standard deviation. Cq, quantification cycle.

Conventional PCR Uniformity

The thermal uniformity of the PTC Tempo Thermal Cyclers was within ±0.4˚C within 10 seconds of arrival at 50, 60, and 90˚C during the thermal validation and thermal qualification process. This uniformity result is equivalent to that of the C1000 Touch Thermal Cyclers.

Conventional PCR uniformity was further assessed using DNA gel electrophoresis and RT-qPCR (SYBR® Green). The C1000 Touch and PTC Tempo Thermal Cyclers demonstrated comparable performance across assays and reaction volumes (Figure 2) and consumables (Figure 3). The PTC Tempo 384 Thermal Cyclers met the criterion of <10% CV for the DNA gel electrophoresis technique.

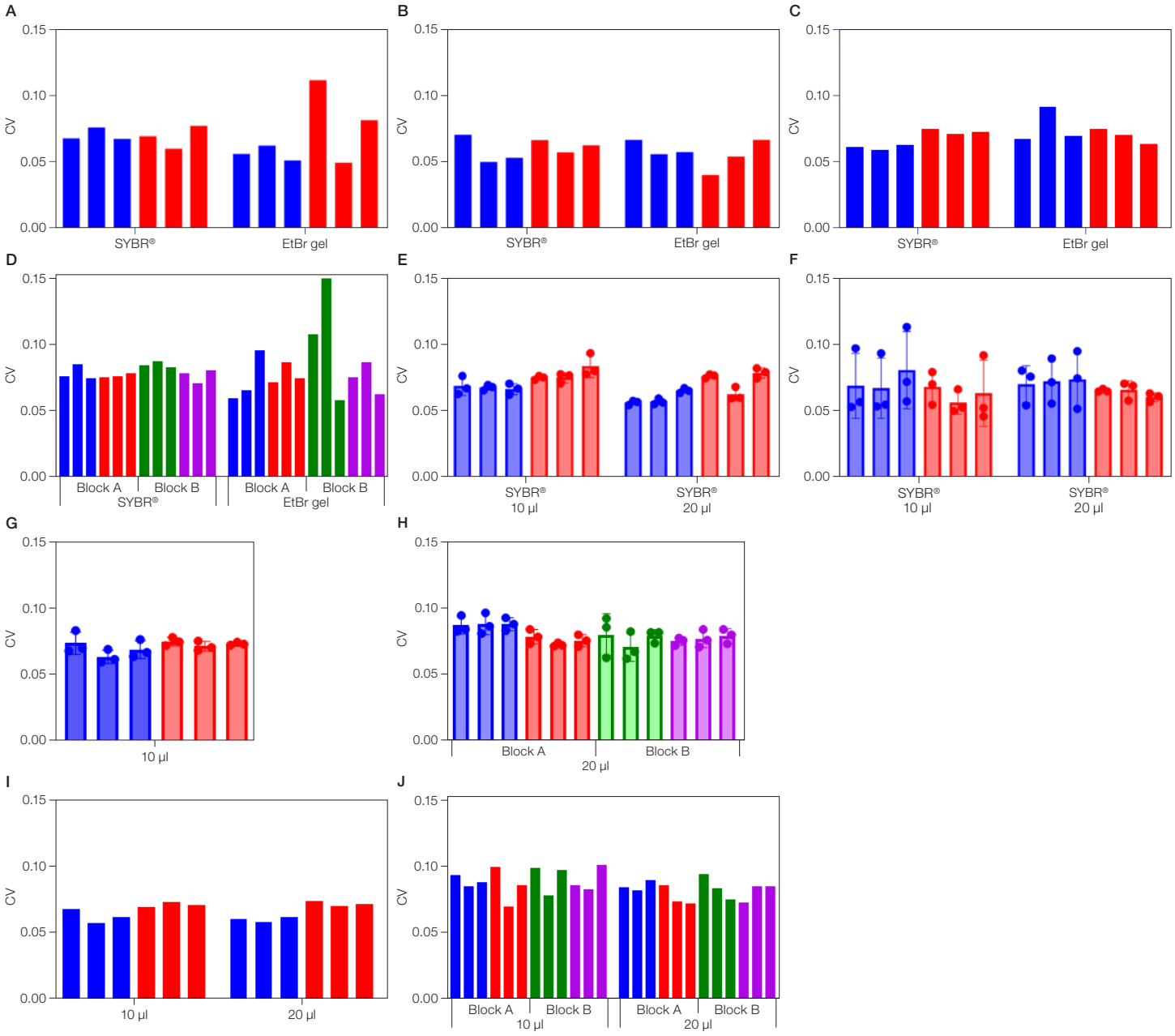


Fig. 2. Conventional PCR uniformity by assay and reaction volume, using RT-qPCR (SYBR® Green) and DNA agarose electrophoresis (EtBr) at high volume. Uniformity was exemplified with less than 10% CV in all but one PTC Tempo Thermal Cycler unit when assessing amplified products across three C1000 Touch and three PTC Tempo Thermal Cyclers. Two C1000 Touch Thermal Cycler units were slightly higher than 10% CV but remained below 15%. **A**, 96-well block format (50 µl of sample); **B**, deep well block format (125 µl of sample); **C**, 384-well block format (20 µl of sample); **D,** 48/48-well block format (50 µl of sample); **E**, 96-well block format (10–20 µl of sample); **F**, deep well block format (10–20 µl of sample); **G**, 384-well block format (10 µl of sample); **H**, 48/48-well block format (20 µl of sample); **I**, 384-well block format (10–20 µl of sample); **J**, 48/48-well block format (10–20 µl of sample). CVs from A–D, I, and J are from one run on the instrument, whereas 2E–H are from three runs on the instrument.

A–C, **E–G**, and **I**, C1000 Touch Thermal Cyclers (■); PTC Tempo Thermal Cyclers (■). **D, H**, and **J**, C1000 Touch Thermal Cycler block A (■); C1000 Touch Thermal Cycler block B (■); PTC Tempo Thermal Cycler block A (■); PTC Tempo Thermal Cycler block B (■).

CV, coefficient of variation.

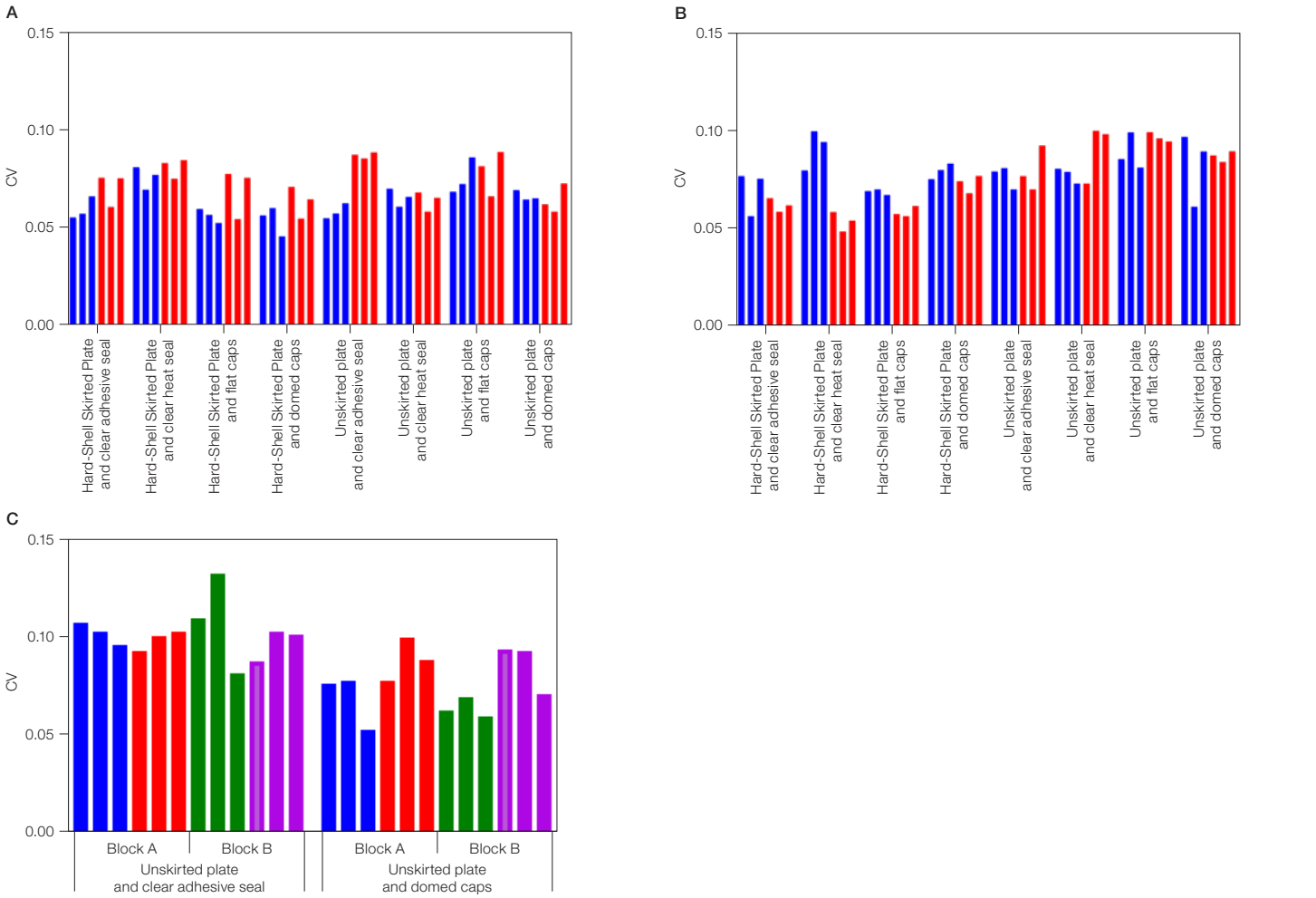


Fig. 3. Conventional PCR uniformity using different consumables. Uniformity was demonstrated with less than 10% CV in all but one C1000 Touch Thermal Cycler unit when assessing amplified products across three C1000 Touch and three PTC Tempo Thermal Cyclers. **A**, 96-well block format (20 µl of sample); **B**, deep well block format (20 µl of sample); **C**, 48/48-well block format (20 µl of sample). The 96-fast and 96–deep well thermal cycler configurations used a unique heat seal for plates: PCR Plate Heat Seal, clear, optical (Bio-Rad, #1814030). In 3A and B, 0.2 ml Flat PCR Tube 8-Cap Strips, optical, ultraclear (Bio-Rad, #TCS0803) and 0.2 ml Domed PCR Tube 8-Cap Strips, clear (Bio-Rad, #TCS0801) were used. In 3C, the following products were used: Multiplate 48-Well PCR Plates, low profile, unskirted, clear (Bio-Rad, #MLL4801), Microseal 'B' PCR Plate Sealing Film, adhesive, optical (Bio-Rad, #MSB1001) and #TCS0801.

A and **B**, C1000 Touch Thermal Cyclers (■); PTC Tempo Thermal Cyclers (■). **C**, C1000 Touch Thermal Cycler block A (■); C1000 Touch Thermal Cycler block B (■); PTC Tempo Thermal Cycler block A (■); PTC Tempo Thermal Cycler block B (■).

CV, coefficient of variation.

Conventional PCR Product Size

All PTC Tempo Thermal Cyclers achieved the expected results for the three differently sized templates (small, medium, and large) (Appendix, Supplementary Figures 1 and 2). The C1000 Touch Thermal Cyclers were used for comparison using an identical cycling protocol and reaction mixture and also produced the small- and medium-sized bands as expected. However, the C1000 Touch Thermal Cyclers did not produce the large product as frequently as any of the PTC Tempo Thermal Cyclers. In summary, the PTC Tempo Thermal Cycler results met or exceeded the C1000 Touch Thermal Cycler results for the 96-well, 96–deep well, 48/48-well, and 384-well models.

Thermal Gradient Uniformity

The PTC Tempo Thermal Cycler can easily be used for temperature gradient optimization studies to determine the best PCR settings for a specific protocol, as demonstrated in Figure 4.

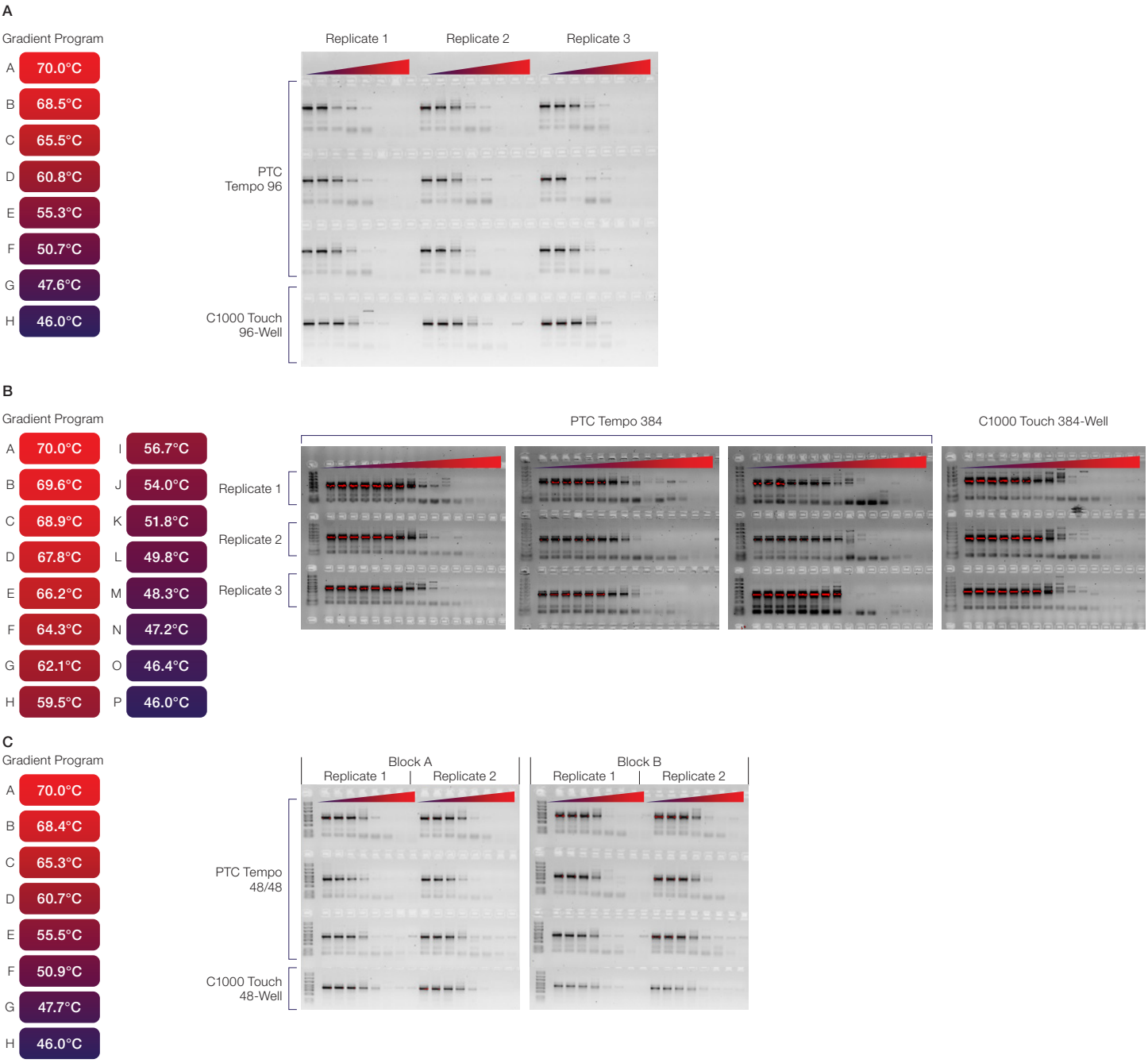


Fig. 4. Representative agarose gels for reaction products obtained using thermal gradients. A, results for the PTC Tempo 96 Thermal Cycler and C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module; **B**, results for the PTC Tempo 384 Thermal Cycler and C1000 Touch Thermal Cycler with 384-Well Reaction Module; **C**, results for the PTC Tempo 48/48 Thermal Cycler and C1000 Touch Thermal Cycler with Dual 48/48-Well Reaction Module.

Table 1. General and system-specific specifications of the PTC Tempo Thermal Cyclers.

Sample block* type	Fixed			
Dimensions (W x D x H)	28 x 50 x 26 cm (11 x 20 x 10 in.)			
Power	100–240 V, 50/60 Hz, 850 W max			
Electrical approvals	IEC, CE			
Memory	4.6 GB; 100,000 files, which include protocols and run reports			
Noise level	50–70 dB at steady state			
Touch-screen display	8 in. LCD			
Programming options	Step-based graphical and automatic			
Security features for regulated environments	Password protection and secure user mode			
Reporting	Exportable run logs and system logs			
Temperature control modes	Calculated and block			
Onboard operating system	Linux			
Communications	WiFi, BR.io cloud platform, USB-A 2.0, Ethernet, USB barcode scanner			
Heating and cooling method	Peltier			
Lid heating	30–110°C			
Temperature range	4–100°C			
Thermal accuracy	±0.2°C of programmed target at 90°C			
Thermal uniformity	±0.4°C well-to-well within 10 sec of arrival at 90°C			
	96-Well	**Deepwell**	**48/48-Well**	**384-Well**
Lid type	Automated	Automated	Nonautomated	Automated
Weight	15 kg (33 lb)	15 kg (33 lb)	14 kg (31 lb)	15 kg (33 lb)
Sample capacity	96 x 0.2 ml tubes or 1 x 96-well plate	96 x 0.2 ml tubes, 48 x 0.5 ml tubes, or 1 x 96-well plate	2 x 48 x 0.2 ml tubes or 2 x 48-well plates	1 x 384-well plate
Sample volume	1–50 µl (10–50 µl recommended)	1–125 µl (10–125 µl recommended)	1–50 µl (10–50 µl recommended)	1–30 µl (3–20 µl recommended)
Maximum ramp rate	5°C/sec	2.5°C/sec	4°C/sec	2.5°C/sec
Average ramp rate	3.3°C/sec	2°C/sec	3°C/sec	2°C/sec
Gradient				
Operational range	30–100°C	30–100°C	30–100°C	30–100°C
Programmable span	8 rows, 1–24°C	8 rows, 1–24°C	8 rows, 1–24°C	16 rows, 1–24°C

API, application programming interface.
* U.S. patents 7,955,573, 8,367,014, 8,557,196, 7,632,464 and 7,051,536.

Sample Block Ramp Rate, Temperature Accuracy, and Uniformity

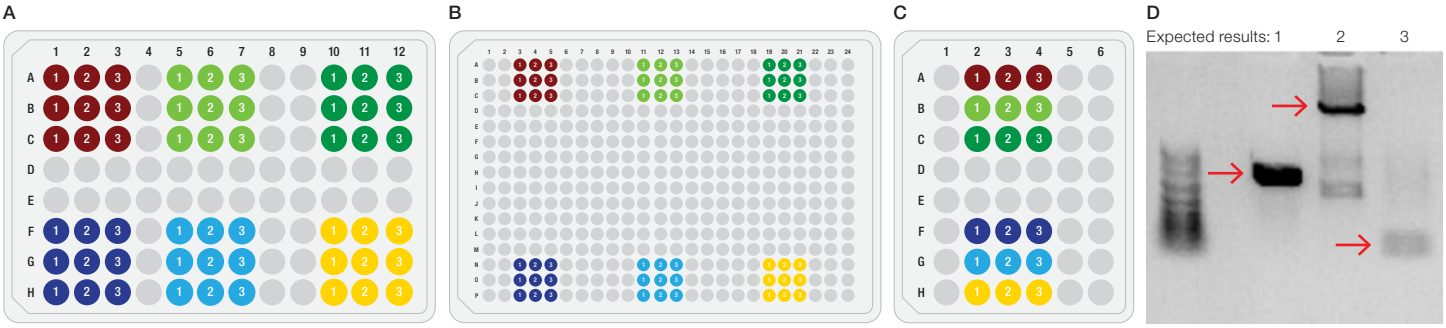
Thermal cycler specifications are summarized in Table 1 and demonstrate that the PTC Tempo Thermal Cyclers are comparable to the C1000 Touch Thermal Cyclers in terms of ramp rate, temperature accuracy, and uniformity.

Conclusions

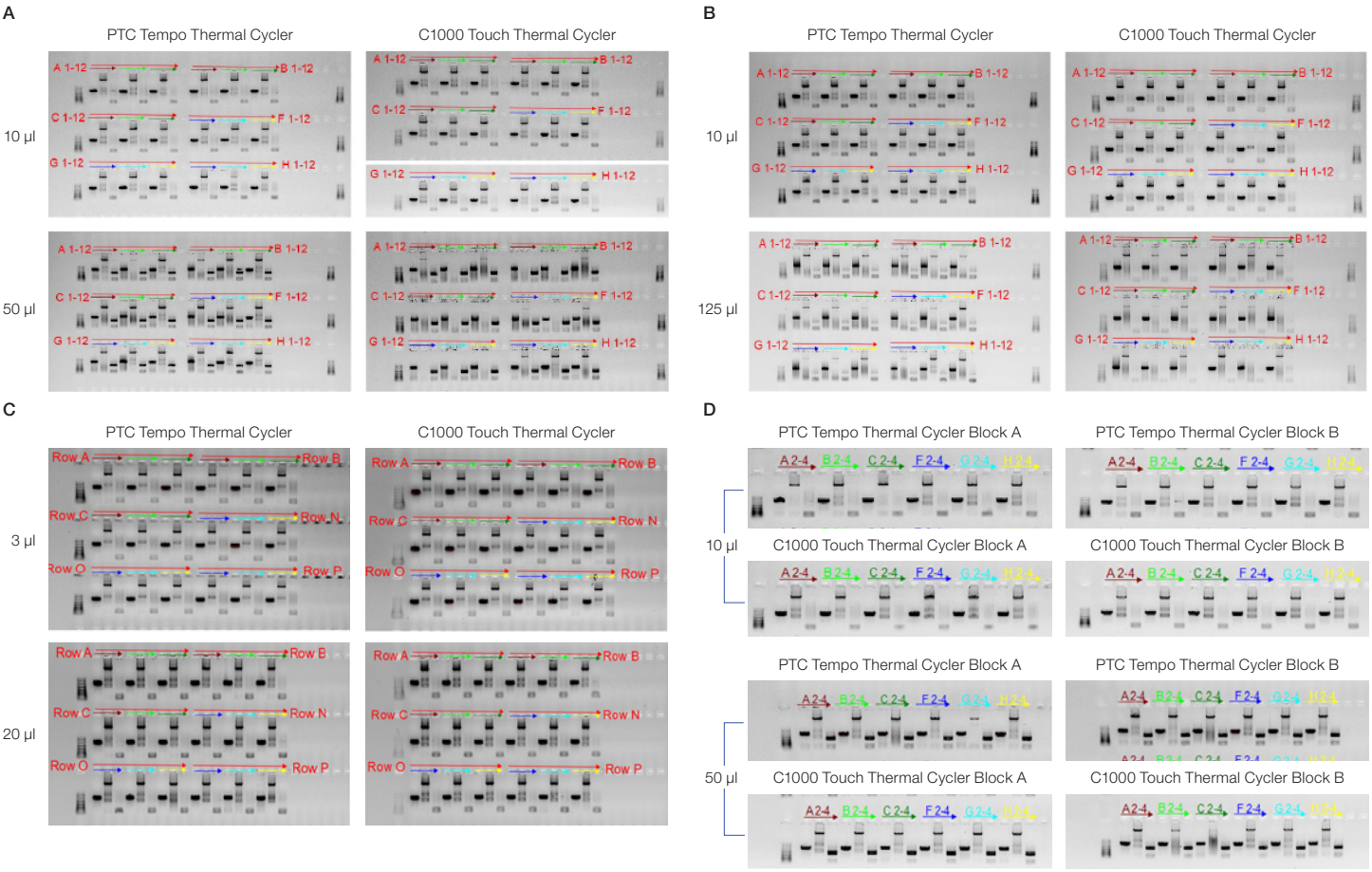
The PTC Tempo 96 and PTC Tempo Deepwell Thermal Cyclers have comparable thermal performance to the C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module and C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module, respectively, in terms of thermal uniformity, accuracy, and ramp rate. Additionally, intra-variability for the PTC Tempo 96 Thermal Cycler is comparable to the C1000 Touch Thermal Cycler with 96-Well Fast Reaction Module, as shown by data from three separate replicates on the block. The PTC Tempo 48/48 Thermal Cycler also has comparable thermal performance to the C1000 Touch Thermal Cycler with Dual 48/48 Fast Reaction Module. Lastly, the PTC Tempo 384 Thermal Cycler performs similarly to the C1000 Touch Thermal Cycler with 384-Well Reaction Module.

Experiments that assess thermal accuracy and uniformity can be repeated in labs that want to perform a bridging study to assess amplified products in a specific downstream application. These procedures can aid in ensuring that PTC Tempo Thermal Cyclers provide reproducible and precise results during the performance of more complex molecular applications.

Appendix: Supplementary Data



Suppl. Fig. 1. Plate layout for evaluating PCR products from Mix 1 (expected size: 1.3 kb), Mix 2 (expected size: 10 kb), and Mix 3 (expected size: 102 bp). **A**, 96-well plate layout; **B**, 384-well plate layout; **C**, 48/48-well plate layout; **D**, expected PCR products.



Suppl Fig. 2. PCR product size analysis for 96-well, deep well, 48/48-well, and 384-well formats. A1–12, B1–12, and C1–12 denote the row and column position of each reaction for the 96-fast and 96–deep well formats. In C, rows A–B, C–N, and O–P show the 384-well format. In D, A2–4, B2–4, C2–4, and so on denote the row and column position of the 48/48-well format. For example, A1–12 denotes the 9 PCR reactions run in a row. **A**, 96-well block format; **B**, deep well block format; **C**, 384-well block format; **D**, 48/48-well block format.

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The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

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