

PCR

PowerFlex Thermal Cycler—technical comparison to the ProFlex PCR System



Figure 1. Features of the Applied Biosystems™ PowerFlex™ Thermal Cycler.

Introduction

The Applied Biosystems™ ProFlex™ PCR System raised the standard for thermal cyclers when it was introduced in 2013. Innovative features included multiple block options with independent control, an LCD touchscreen, a thermal block with multiple

temperature zones, and web-enabled remote-control software. The PowerFlex Thermal Cycler builds on this legacy with the latest block technology and enhanced connectivity options, offering the same high-end performance with improved features (Figure 1).

These advancements permit higher ramp rates, more precise thermal block accuracy and uniformity (Table 1), and a more intuitive user interface for easy protocol setup and monitoring. This study demonstrates that the PowerFlex Thermal Cycler delivers equivalent reliability, accuracy, and consistency in amplification uniformity as the ProFlex PCR System by comparing coefficient of variation (CV) of amplification results across a variety of PCR consumables used.

Table 1. Specification comparison.

Feature	ProFlex PCR System	PowerFlex Thermal Cycler
Block format	96-well 3 x 32-well 2 x 384-well 2 x 96-well Dual flat	96-well 3 x 32-well 2 x 384-well
Block type	Aluminum	Aluminum
Max. block ramp rate	6°C/sec	7°C/sec
Temperature accuracy	±0.25°C (35–99°C)	±0.15°C (35–99°C)
Temperature uniformity	<0.5°C (at 95°C)	<0.4°C (at 95°C)
Temperature range	0–100.0°C	0–100.0°C
Dimensions (H x W x D)	33 x 56.5 x 27.2 cm	30.3 x 51.5 x 25.8 cm

Note: These specifications are for 96-well and 3 x 32-well block formats.

PowerFlex Thermal Cycler highlights

- **Higher ramp rates**—enable faster time-to-results
- **Precise thermal control**—designed to meet more stringent thermal control requirements, featuring an accuracy of ±0.15°C and uniformity of <0.4°C (at 95°C) and <0.3°C (at 72°C)
- **Simulation mode**—allows users to select from a list of instruments, including the ProFlex PCR System, for easy instrument transition and consistency in experimental protocols
- **Compatibility with all PCR plates**—96-well block format is compatible with fully skirted, semi-skirted, and non-skirted plates
- **Enhanced user interface**—expanded 10.1-inch touchscreen and improved user interface for an intuitive, interactive experience with easy programming
- **Smart Help**—fast and accurate troubleshooting directly from the instrument

Materials and methods

To verify the performance of the PowerFlex Thermal Cycler, a variety of Applied Biosystems™ PCR plastics was used during testing. A complete list of Applied Biosystems™ MicroAmp™ consumables used in this study is shown in Table 2. All PCR plate consumables were tested using the ProFlex PCR System, 96-well (Cat. No. 4484075), ProFlex PCR System, 3 x 32-well (Cat. No. 4484073), Applied Biosystems™ Automated Thermal Cycler, 96-well (Cat. No. A31491), PowerFlex Thermal Cycler, 96-well (Cat. No. A40008064), and PowerFlex Thermal Cycler, 3 x 32-well (Cat. No. A40008063).

Table 2. PCR consumables used in amplification uniformity testing.

Description	Cat. No.
MicroAmp EnduraPlate Optical 96-Well Clear Reaction Plates with Barcode	4483354
MicroAmp EnduraPlate Optical 96-Well Full-Skirted Plates with Barcode	A31728
MicroAmp 8-Tube Strip, 0.2 mL, standard profile, clear, separate caps required	N8010580
MicroAmp 8-Tube Strip with Attached Domed Caps, 0.2 mL, standard profile, clear	A30589
MicroAmp Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL, standard profile, clear	A30588
MicroAmp Optical 96-Well Reaction Plate	N8010560
MicroAmp Reaction Tube with Cap, 0.2 mL	N8010540
MicroAmp Reaction Tube without Cap, 0.2 mL	N8010533
MicroAmp TriFlex 3 x 32-Well PCR Reaction Plate	A32810

For amplification uniformity testing, 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'-GATGAGTTCGTGTCCGTACAAC-3') and lambda reverse primer (5'-ACGGCTGCACGGAGTTCAGTATG-3') at 0.2 μM concentration. The bulk reaction was tested at 10 μL or 100 μL volume for 96-well reactions using different PCR consumables on the ProFlex PCR System and the PowerFlex Thermal Cycler. The following thermal profile was used: 95°C for 10 min, 25 cycles at 94°C for 15 sec and 69.5°C for 90 sec, and a final stage at 72°C for 7 min. Upon completion of thermal cycling, the PCR reactions were transferred to the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (Cat. No. A28139) for

fluorometric analysis. Amplification uniformity was calculated as CV using the following formula:

$$CV (\%) = \frac{\text{Standard deviation of SYBR Green dye signal for each run}}{\text{Average SYBR Green dye signal for each run}} \times 100\%$$

For robustness testing, short-amplicon reactions were prepared using Applied Biosystems™ AmpliTaq Gold™ 360 Master Mix (Cat. No. 4398886) according to the standard protocols with human genomic DNA (MilliporeSigma, Cat. No. 11691112001). Long-amplicon reactions were prepared using the Applied Biosystems™ SequalPrep™ Long PCR Kit with dNTPs (Cat. No. A10498) according to the standard protocol. Short-amplicon reactions were tested at 10 µL volume and long-amplicon reactions were tested at 20 µL volume to compare different PCR consumables on the ProFlex PCR System and PowerFlex Thermal Cycler. Gel electrophoresis was performed after PCR with the Invitrogen™ E-Gel™ Power Snap Electrophoresis Device (Cat. No. G8100) to separate targets according to size. Gels were visualized using the Invitrogen™ iBright™ FL1500 Imaging System (Cat. No. A44241).

Results

Amplification uniformity

CV was calculated using fluorescence intensity data from either one ProFlex PCR System or Automated Thermal Cycler (ATC) and three PowerFlex Thermal Cyclers. Figure 2 shows amplification uniformity across a 96-well plate, for two reaction volumes, 10 µL (low) and 100 µL (high); Figure 3 shows amplification uniformity across a 3 x 32-well format comparing all 3 blocks on each instrument for 10 µL and 100 µL reaction volumes. Figures 4 and 5 show thermal cycler uniformity on 96-well and 3 x 32-well instruments, respectively, evaluated across various combinations of PCR plates, adhesive films, tubes, tube strips, and cap strips by measuring the average fluorescence intensity of 10 µL PCR reactions. Run-to-run amplification uniformity on a 96-well and 3 x 32-well instrument is demonstrated in Figures 6 and 7, respectively, showing the PowerFlex Thermal Cycler supports reproducible thermal performance across separate 10 µL PCR reactions. Overall, the results show comparable well-to-well and run-to-run uniformity between thermal cyclers using different reaction volumes and PCR consumables. The results were reproducible across all PowerFlex Thermal Cyclers tested.

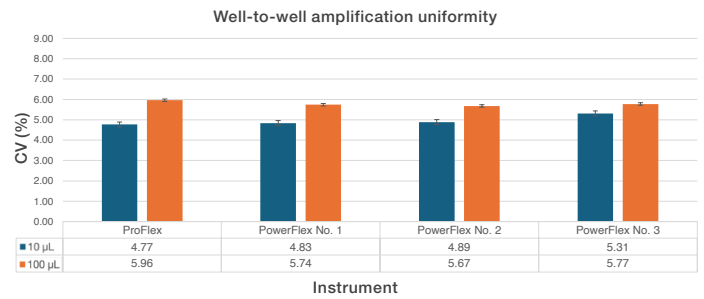


Figure 2. Comparison of well-to-well amplification uniformity between one ProFlex 96-Well PCR System and three PowerFlex 96-Well Thermal Cyclers at low (10 µL) and high (100 µL) reaction volumes. The CV for each run was calculated using fluorescence intensity data after PCR in a MicroAmp Optical 96-Well Reaction Plate sealed with Applied Biosystems™ MicroAmp™ Clear Adhesive Film.

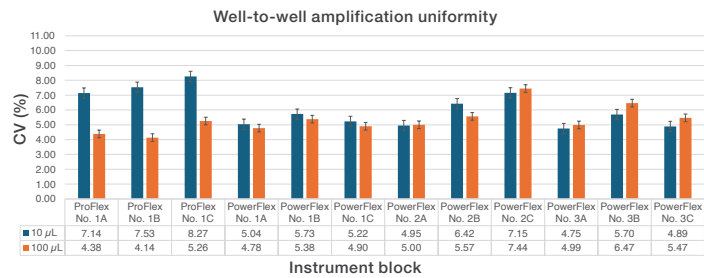


Figure 3. Comparison of well-to-well amplification uniformity between one ProFlex 3 x 32-Well PCR System and three PowerFlex 3 x 32-Well Thermal Cyclers at 10 µL reaction volumes. The CV for each run was calculated using fluorescence intensity data after PCR in a MicroAmp EnduraPlate PCR plate sealed with MicroAmp Clear Adhesive Film.

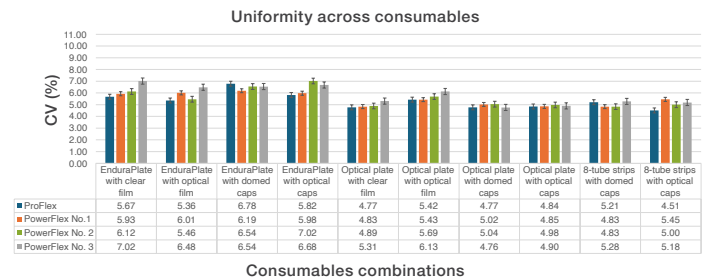


Figure 4. Comparison of well-to-well amplification uniformity between one ProFlex 96-Well PCR System and three PowerFlex 96-Well Thermal Cyclers using various combinations of PCR consumables including MicroAmp EnduraPlate PCR plates, optical plates, and 8-tube strips. The CV for each run was calculated using fluorescence intensity data from low-volume (10 µL) reactions.

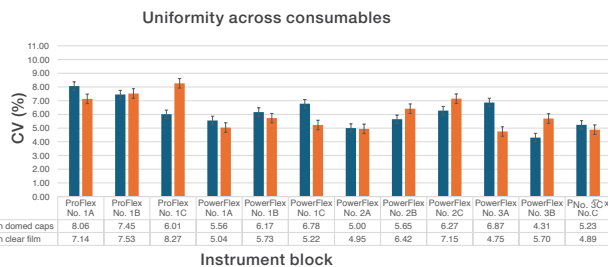


Figure 5. Comparison of well-to-well amplification uniformity between one ProFlex 3 x 32-Well PCR System and three PowerFlex 3 x 32-Well Thermal Cyclers using MicroAmp TriFlex plates with domed or clear caps. The CV for each run was calculated using fluorescence intensity data from 10 μ L reactions.

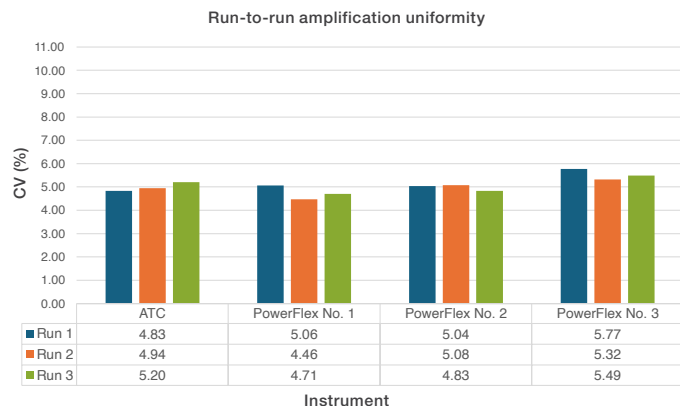


Figure 6. Comparison of run-to-run amplification uniformity between one ATC and three PowerFlex 96-Well Thermal Cyclers on MicroAmp EnduraPlate Optical 96-Well Full-Skirted Plates with clear film. The CV for each run was calculated using fluorescence intensity data after PCR in 10 μ L reactions.

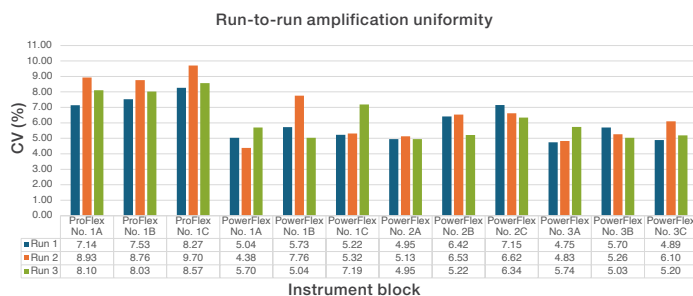


Figure 7. Comparison of run-to-run amplification uniformity between one ProFlex 3 x 32-Well PCR System and three PowerFlex 3 x 32-Well Thermal Cyclers using MicroAmp TriFlex 3 x 32-Well plates with 32-well clear adhesive film. The CV for each run was calculated using fluorescence intensity data after PCR in 10 μ L reactions.

Robust thermal performance

The PowerFlex Thermal Cycler demonstrates robust performance by amplifying targets of different lengths and complexities, including standard DNA sequences, high AT- or GC-rich sequences, and amplicons likely to form primer-dimers. Successful amplification of the targets was demonstrated by the presence of distinct bands of correct sizes after gel electrophoresis. The results in Figure 8 show that the PowerFlex Thermal Cycler supports thermal robustness equivalent to the ProFlex PCR System without the need to reoptimize experiments.

A



B

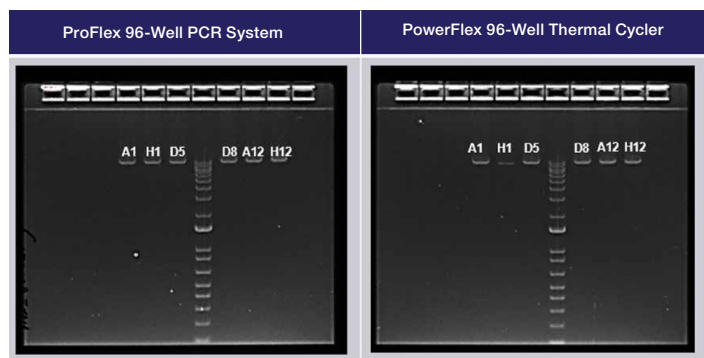


Figure 8. Comparison of robustness between the ProFlex 96-Well PCR System and the PowerFlex 96-Well Thermal Cycler. (A) AmpliTaq Gold 360 Master Mix was used to amplify single, short human genomic DNA fragments (313 bp, 499 bp, 556 bp, or 585 bp) of different complexities in MicroAmp 8-Tube Strips with Attached Domed Caps. **(B)** The SequelPrep Long PCR Kit was used to amplify a single, long human genomic DNA amplicon (12,342 bp) in MicroAmp 8-Cap Strips.

Easy temperature optimization

The PowerFlex Thermal Cycler features Applied Biosystems™ VeriFlex™ Blocks technology, utilizing multiple Peltier elements to optimize PCR temperature with precision (Figure 9). Users can set temperatures across the block based on minimum and maximum

temperatures, set the midpoint, or provide more precise control using the 6 temperature zones. With 6-zone control, users can set up to a maximum difference of 10°C between zones with a 30°C maximum range across the VeriFlex Blocks.

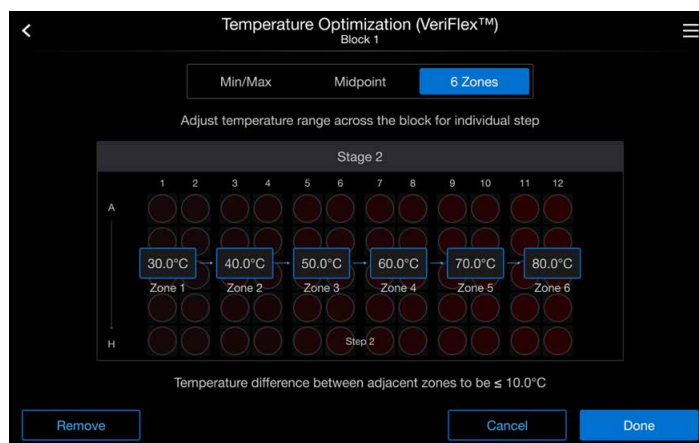
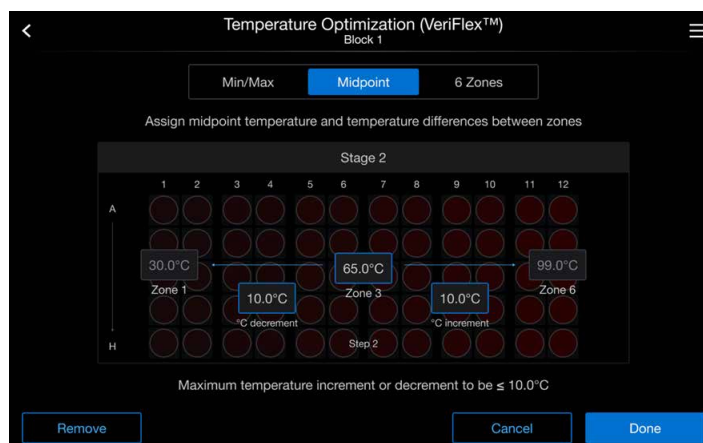
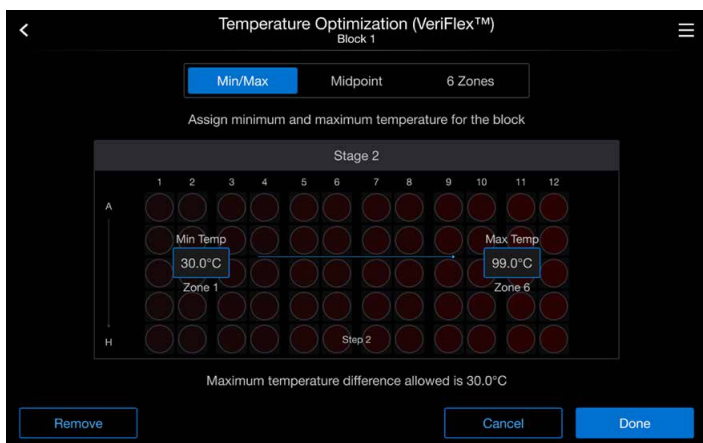


Figure 9. User interface of the PowerFlex 96-Well Thermal Cycler for temperature optimization.

Easy protocol transition

For users concerned about reoptimizing protocols to run on the PowerFlex Thermal Cycler, simulation modes eliminate the need to do this work. Simulation modes allow the user to run protocols under the same conditions as a different thermal cycler. The user selects from a list of instruments, including the ProFlex PCR System, for easy instrument transition and consistency in experimental protocols.

Secure remote access

The PowerFlex Thermal Cycler is a cloud-enabled instrument, allowing users to connect anytime, anywhere with a mobile device or desktop computer. Access is secure and private with an account on the Thermo Fisher™ Connect Platform to design and share protocols, download experimental report files, schedule an instrument run, or check run status remotely.

Multi-instrument control

The PowerFlex Thermal Cycler has multi-instrument run options, enabling one PowerFlex instrument on the same network to run the same protocol on many instruments simultaneously.

Smart Help

The Smart Help functionality allows users to submit service or technical support tickets directly from the thermal cycler (Figure 10). Log files and run history can be automatically attached, enabling faster issue diagnosis and resolution.

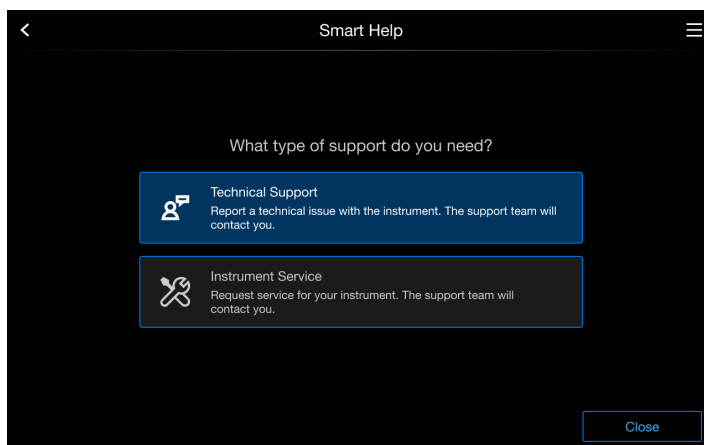


Figure 10. User interface for submitting support tickets through the Smart Help functionality.

Reliability

Each PowerFlex Thermal Cycler comes with the reliability and quality associated with Applied Biosystems™ instruments. Components used to manufacture thermal cyclers must pass rigorous testing for repeated stress, environmental conditions, including low and high temperature and humidity, as well as shock and vibration testing according to International Safe Transit Association (ISTA) standards. This helps ensure each PowerFlex unit delivers high-quality performance.

Conclusion

The comparative analysis demonstrates that the PowerFlex Thermal Cycler delivers performance equivalent to the ProFlex PCR System, while offering significant improvements in speed, precision, and usability. Across all evaluations—including amplification uniformity, run-to-run reproducibility, and robustness—the PowerFlex system consistently produced reliable, high-quality results. The PowerFlex Thermal Cycler upholds the trusted reliability of Applied Biosystems instruments while exhibiting advanced technologies that streamline PCR workflows and enhance operational efficiency—all without compromising data quality.