VeriFlex temperature control technology for thermal cycling

Introduction

Temperature cycling is the basis for all PCR, and how your thermal cycler performs is of critical importance to you and your research. Temperature, ramp rate, and hold time are the critical parameters controlled by a thermal cycler for a successful PCR reaction. A high-performing thermal cycler must have the ability to precisely control the liquid (reaction) temperature and hold time, regardless of the reaction volume. The temperature and hold-time control of a thermal cycler is even more critical during PCR optimization experiments. The thermal cycler must provide the same performance during normal PCR and during PCR optimization runs. This paper analyzes the construction of instruments that use Applied Biosystems™ VeriFlex™ Blocks temperature control technology or conventional gradient blocks, and compares their performance.

What is gradient temperature control?

Gradient temperature control is one of the features that helps the user conduct PCR optimization experiments to determine the optimum temperature and hold time of a PCR protocol, and do it with the minimal number of experiments. Ideally, a true gradient will exhibit a linear temperature slope across a homogeneous metal block, as shown in Figure 1.

Gradient thermal cyclers on the market today are constructed with two separate heating/cooling elements below a homogeneous metal block, as shown in Figure 2. This design results in the following limitations:

- On a gradient thermal cycler, the user can only set two temperatures and cannot control other temperatures across the block
- A true linear gradient is not achievable because of heat interactions between the high and low temperature set at each end of the homogeneous metal block; instead of a linear gradient, the temperature across a homogeneous metal block follows more of a sigmoidal curve, as shown in Figure 3

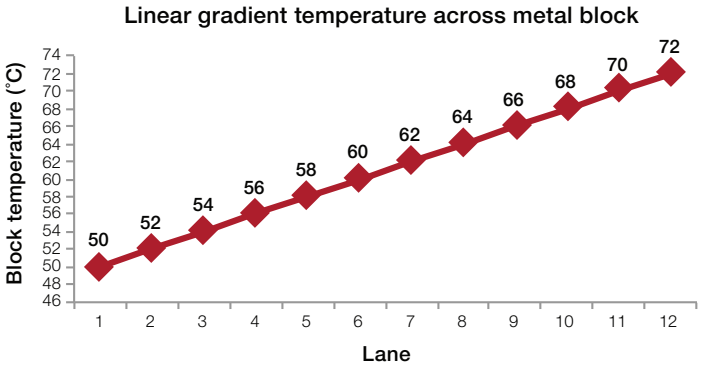

Figure 1. Linear gradient temperature.

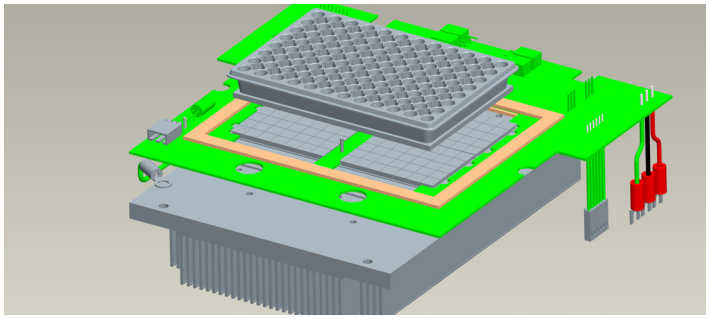

Figure 2. Gradient thermal cycler construction.

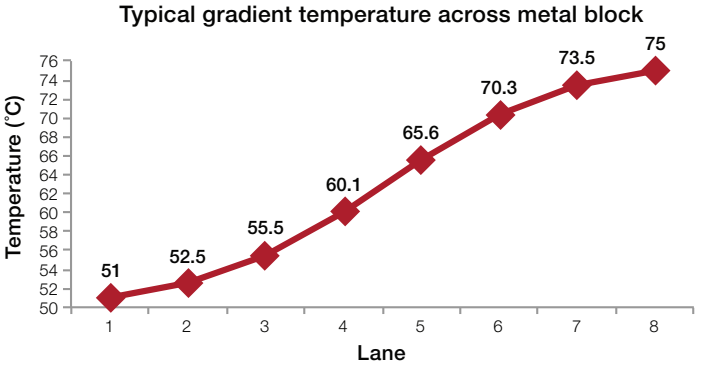

Figure 3. Nonlinear gradient temperature.

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What is VeriFlex Blocks temperature control?

Thermal cyclers that use VeriFlex technology are constructed with 3 or more separate heating/cooling elements below each of the 3 or more segmented metal blocks, as shown in Figure 4. Each pair of heating/cooling elements and segmented metal blocks are completely insulated from each other to help prevent heat interactions. As a consequence, VeriFlex technology provides a true linear temperature slope across metal blocks, as shown in Figure 1, with the ability to set up to 6 different temperatures. The user can set each temperature zone uniquely, allowing for better control of temperature optimization.

VeriFlex Blocks technology compared to conventional gradients

Materials and methods

The instruments in Table 1 were all tested using the same equipment and methods, which are described in this section.

Table 1. Instruments tested.

Model name	Cat. No.
Eppendorf™ Mastercycler™ Nexus Gradient	6331 000.017
Eppendorf™ Mastercycler™ Nexus GX2	6336 000.015
Bio-Rad™ C1000 Touch™ 96-well Fast	185-1196
Bio-Rad™ T100™	186-1096
Applied Biosystems™ ProFlex™ 1 x 96 well	4484075
Applied Biosystems™ SimpliAmp™	A24811
SensoQuest Labcycler	011-101, 012-103
Bioer GeneMax	BYQ6067
TaKaRa™ Dice™ Touch	TP 350

Block temperature was measured using an NIST-traceable Applied Biosystems™ VeriFlex™ 96-well Temperature Verification Kit (Cat. No. 4377669) with 3 temperature probes. Each probe measures temperature at 8 positions, providing a total of 24 temperature measurements at various points on the block. An example of the temperature probe layout is diagrammed in Figure 5.

Temperatures are the average probe temperatures of each row, column, or zone.

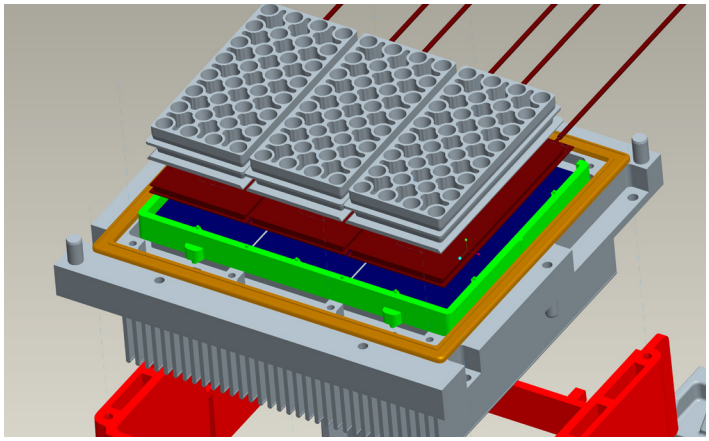

Figure 4. VeriFlex technology construction.

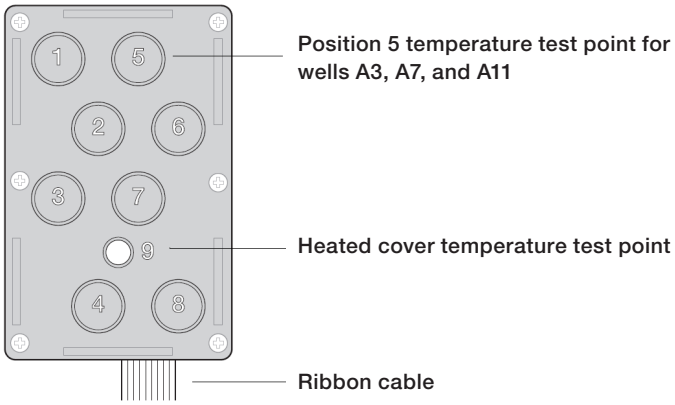

Figure 5. VeriFlex Temperature Verification Kit temperature probe layout.

VeriFlex Blocks technology versus conventional gradient block accuracy

Each instrument in this study was tested using its gradient or VeriFlex Blocks technology to vary temperatures. 60°C (a common annealing temperature) was used as the approximate center point, and then each block was tested with the maximum temperature range allowed by the instrument. Data from several temperature settings, actual measurements, and calculated differences are shown in Table 2.

Table 2. Example accuracy at various set points over a 20°C range.

Temperature set point* (°C)	50.5	51.7	53.6	56.2	58.8	61.2	63.8	66.4	68.3	69.5
Block temp. measurement** (°C)	50.6	51.8	53.7	56.1	58.7	61.2	63.8	66.3	68.4	69.4
Delta† (°C)	0.1	0.1	0.1	–0.1	–0.1	0.0	0.0	–0.1	0.1	–0.1

* As displayed on the interface of the thermal cycler.

** As measured using the VeriFlex 96-well Temperature Verification Kit.

† See Table 3 for the largest discrepancy observed across the whole temperature range for each instrument tested.

Results

Measured temperature profiles across the metal blocks of each thermal cycler are shown in Figure 6. VeriFlex Blocks temperature profiles show true linear temperature control across the blocks. All gradient blocks show a similar nonlinear temperature profile (red line) that gives inconsistent lane-to-lane temperature differences (blue bars), except the Bioer GeneMax thermal cycler. The inconsistent temperature differences from lane-to-lane make it challenging to determine the true optimized annealing temperature, especially when optimizing difficult assays.

The published values for temperature ranges and the number of different temperatures available for the instruments in this study, along with the maximum discrepancy between set points and the measured temperatures for each instrument, are shown in Table 3.

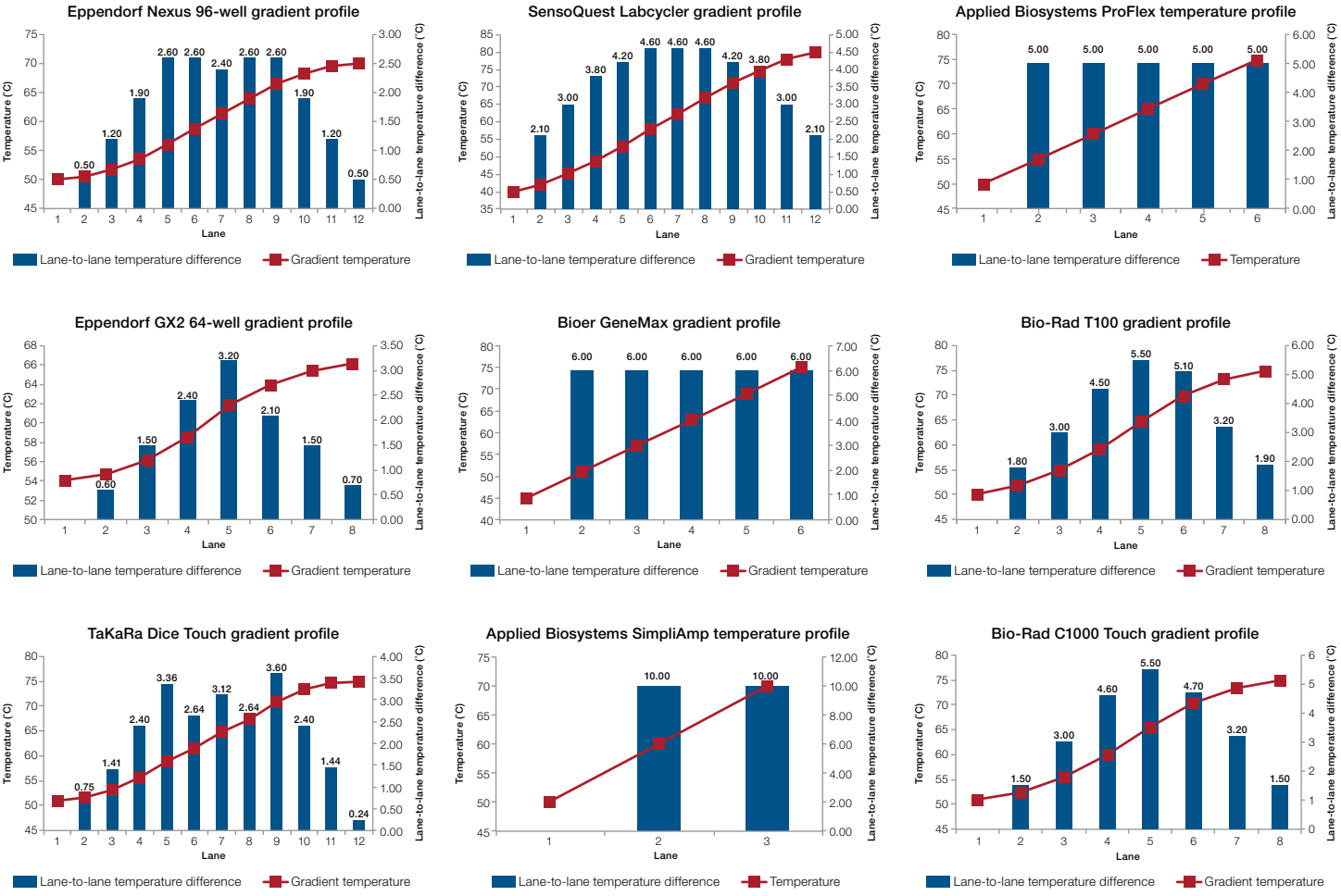

Figure 6. Measured temperature profiles across thermal cycler metal blocks.

Table 3. Temperature accuracy of conventional gradients compared to VeriFlex Blocks technology.

Instrument	Temperature range	Number of different temperatures in span	Accuracy (maximum discrepancy, set point vs. actual)
Bio-Rad C1000 Touch	24°C	8	–1.2°C at 75°C
Bio-Rad T100	25°C	8	+0.6°C at 50°C
SensoQuest Labcycler	40°C	12	–0.9°C at 66.9°C
Eppendorf Mastercycler Nexus Gradient	20°C	12	–0.2°C at 58.8°C
Eppendorf Mastercycler Nexus GX2	12°C	8	–0.4°C at 61.8°C
Bioer GeneMax	30°C	6	+0.3°C at 69°C
Applied Biosystems ProFlex	25°C	6	–0.2°C at 75°C
Applied Biosystems SimpliAmp	20°C	3	0.0 at 60°C
TaKaRa Dice Touch	24°C	12	–0.8°C at 58.9°C

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Summary

Conventional gradients	VeriFlex Blocks technology
One homogeneous metal block	Multiple segmented metal blocks
Only two temperature set points, at both ends of single metal block	Temperatures can be set for each segmented metal block
Temperature control at both ends of metal block	Independent and precise temperature control of each segmented metal block
Temperatures across metal block are nonlinear	Temperature across multiple segmented metal blocks can be linear
Temperature hold time varies across metal block during gradient experiments	Designed to set temperature hold times accurately
Difficult to resolve small temperature differences during gradient experiments	Enables independent and precise temperature control of each segmented metal block; easily permits resolution of temperature differences of 0.1°C
Temperature accuracy in gradient mode may not match the temperature accuracy of normal mode	Temperature accuracy maintained whether in VeriFlex Blocks mode or normal mode

Additional VeriFlex Blocks technology applications

VeriFlex Blocks provide a better-than-gradient approach to PCR optimization. VeriFlex Blocks come in multiple zonal Peltier blocks that are controlled individually by a temperature controller. Each Peltier block is physically isolated using heat insulator material to minimize temperature interference from adjacent Peltier blocks. The result enables precise control over multiple temperature zones.

- Offers highly accurate determination of optimal annealing temperature to help eliminate guesswork
- Helps save time and offers the ability to run multiple reactions with different annealing temperatures in a single PCR run
- Use your thermal cycler as an incubator with multiple precise incubation temperatures

Find out more at **thermofisher.com/thermalcyclers**

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