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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 are even more critical during PCR optimization experiments. The thermal cycler must provide the same performance during normal PCR and during PCR optimization runs. This study analyzes the construction of thermal cyclers that use either 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 optimal temperature and hold time for a PCR protocol in the fewest experiments possible. 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 has 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.

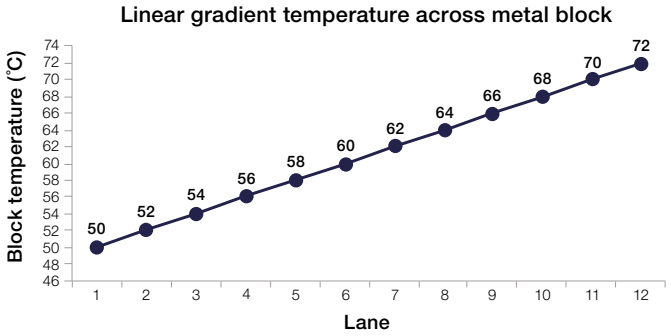

Figure 1. Linear gradient temperature.

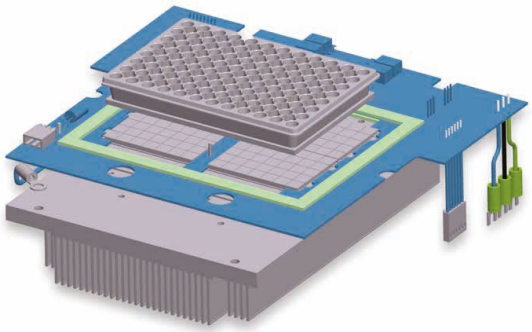

Figure 2. Gradient thermal cycler construction.

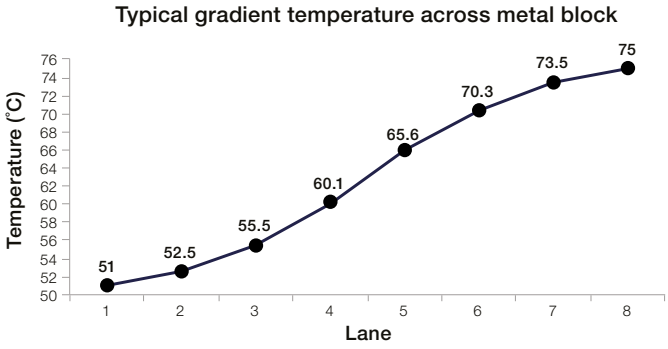

Figure 3. Nonlinear gradient temperature.

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What is VeriFlex Blocks temperature control?

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

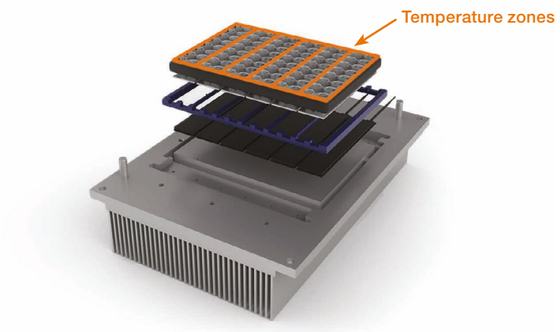

Figure 4. VeriFlex technology construction.

VeriFlex Blocks technology compared to conventional gradients

Materials and methods

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

Table 1. Thermal cyclers tested.

Model name	Cat. No.
Bio-Rad C1000™ Touch	185-1196
Bio-Rad T100™	186-1096
Bioer Life ECO	BYQ6078
Eppendorf™ Mastercycler™ X50	6311 000.010
TaKaRa™ Dice™ Touch	TP350
Applied Biosystems™ ProFlex™ 1 x 96-Well	15137105
Applied Biosystems™ VeritiPro™ 1 x 96-Well	16893330
Applied Biosystems™ SimpliAmp™	15224438
Biometra TAdvanced	846-x-070-201
Biometra TOne	846-x-070-301

Block temperature was measured using a National Institute of Standards and Technology (NIST)–traceable Applied Biosystems™ VeriFlex™ 96-Well Temperature Verification Kit (Cat. No. 16514811) with three temperature probes. Each probe measured temperature at eight 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.

The probe temperatures of each row, column, or zone were averaged to determine the measurements.

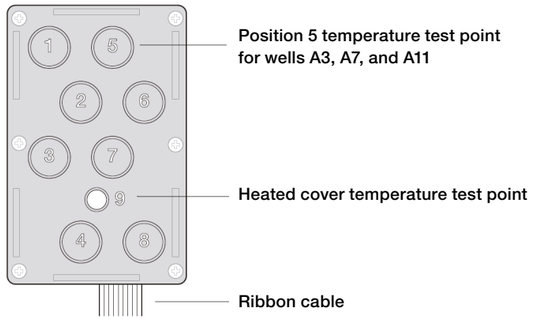

Figure 5. VeriFlex Temperature Verification Kit temperature probe layout.

VeriFlex Blocks technology compared to conventional gradient block accuracy

Each thermal cycler in this study was tested using its gradient or VeriFlex Blocks technology to vary temperature. 60°C (a common annealing temperature) was used as the approximate center point, and each block was tested with the maximum temperature range allowed by the instrument. Data for 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 temperature measurement** (°C)	50.6	51.8	53.7	56.1	58.7	61.2	63.8	66.3	68.4	69.4
ΔT† (°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 thermal cycler tested.

Results

Temperature profiles measured across the metal blocks of the thermal cyclers are shown in Figure 6. The VeriFlex Blocks temperature profiles show true linear temperature control across the blocks. All gradient blocks show similar nonlinear temperature profiles (blue line) that give inconsistent lane-to-lane temperature differences (blue bars). Inconsistent temperature differences from lane to lane make it challenging to determine the true optimal annealing temperature, especially when optimizing difficult assays.

The published values for the temperature ranges, the number of different temperatures available for the thermal cyclers in the study, the maximum discrepancies between set points, and the measured temperatures for each instrument are shown in Table 3.

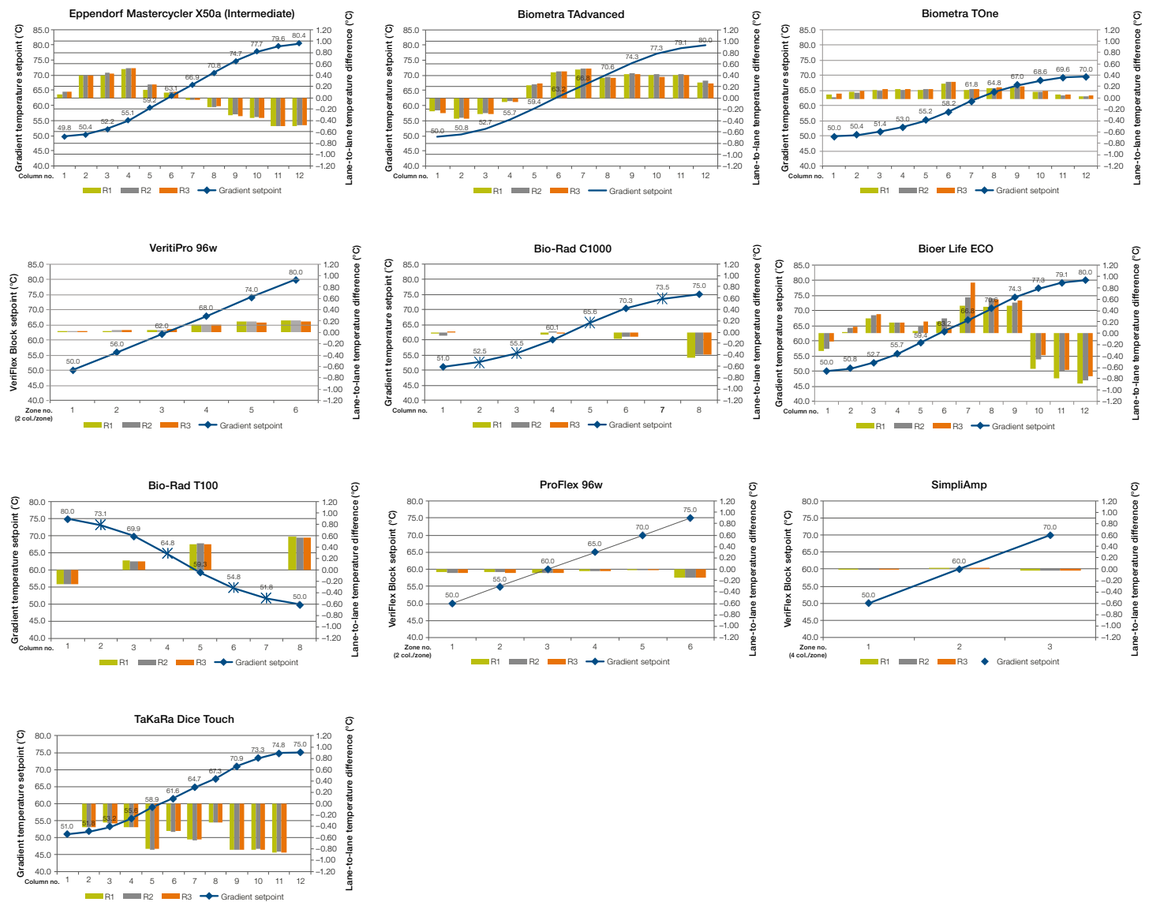

Figure 6. Measured temperature profiles across thermal cycler metal blocks.

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

Thermal cycler	Maximum temperature range in VeriFlex mode or gradient mode	No. of different temperatures in VeriFlex mode or gradient mode	Accuracy (maximum discrepancy (ΔT) between set point and actual measurement)
Bio-Rad C1000 Touch	24°C	8	±1.2°C at 75°C
Bio-Rad T100	25°C	6	±0.6°C at 50°C
Bioer Life ECO	30°C	12	±0.1°C at 55°C
Mastercycler X50	30°C	12	±0.15°C at 55°C
TaKaRa Dice Touch	24°C	12	±0.8°C at 58.9°C
ProFlex 1 x 96-Well	25°C	6	±0.2°C at 75°C
VeritiPro 1 x 96-Well	30°C	6	±0.25°C at 35–99.9°C
SimpliAmp	20°C	3	0.0°C at 60°C
Biometra TAdvanced	30°C	6	±0.20°C at 55°C
Biometra TOne	20°C	12	±0.20°C at 55°C

Summary

Conventional gradients	VeriFlex Blocks technology
One homogeneous metal block	Multiple segmented metal blocks
Only two temperature set points at both ends of the metal block	Temperatures can be set for each segmented metal block
Temperature control at both ends of the 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 in 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 by heat insulating 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
- Thermal cycler can be used as an incubator with multiple precise incubation temperatures

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22457